Bioresource Technology 214 (2016) 303–310

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

High level extracellular production of a recombinant alkaline catalase in *E. coli* BL21 under ethanol stress and its application in hydrogen peroxide removal after cotton fabrics bleaching

Zhenxiao Yu [1], Hongchen Zheng [1], Xingya Zhao, Shufang Li, Jianyong Xu, Hui Song *

Industrial Enzymes National Engineering Laboratory, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China
Tianjin Key Laboratory for Industrial Biological Systems and Bioprocessing Engineering, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China

HIGHLIGHTS

- The yield of alkali catalase (KatA) in *E. coli* was the highest level reported so far.
- The yield of recombinant KatA was significantly enhanced by 2% ethanol stress.
- Triton X-100 supplementation could markedly improved extracellular ratio of KatA.
- KatA shows higher H_2O_2 removal rate and lower cost than traditional process.

ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 21 April 2016
Accepted 22 April 2016
Available online 26 April 2016

Keywords:
Alkaline catalase
Ethanol stress
Triton X-100
Fed-batch fermentation
Textile applications

ABSTRACT

The effects of induction parameters, osmolytes and ethanol stress on the productivity of the recombinant alkaline catalase (KatA) in *Escherichia coli* BL21 (pET26b-*KatA*) were investigated. The yield of soluble KatA was significantly enhanced by 2% ethanol stress. And a certain amount of Triton X-100 supplementation could markedly improved extracellular ratio of KatA. A total soluble catalase activity of 78,762 U/mL with the extracellular ratio of 92.5% was achieved by fed-batch fermentation in a 10 L fermentor, which was the highest yield so far. The purified KatA showed high stability at 50 °C and pH 6–10. Application of KatA for elimination of H_2O_2 after cotton fabrics bleaching led to less consumption of water, steam and electric power by 25%, 12% and 16.7% respectively without productivity and quality losing of cotton fabrics. Thus, the recombinant KatA is a promising candidate for industrial production and applications.

1. Introduction

Textile industry is a traditional pillar industry in developing countries. Although it possesses a considerable proportion of the total industrial production value, problems on pollution and water consumption (100 L/kg fabric) have restricted its development in a large extent. Hydrogen peroxide (H_2O_2) is usually used to perform the bleaching process at an alkaline condition in textile industry. The residual H_2O_2 in both the subsequent dying process and downstream decontamination of wastewater is the main hinder needing to be eliminated (Arslan-Alaton et al., 2012). In traditional way, H_2O_2 is removed by washing with large amount of hot water (⩾85 °C), which causes waste in both water and energy (Fruhwirth et al., 2002). In recent years, biodegradation of hydrogen peroxide by alkaline catalase, an economical and environment friendly method, has been supposed to be a promising choice in textile industry (Muster et al., 2015).

Catalase (CAT), typically heme-containing and homotetrameric, is an antioxidant enzyme that catalyze the degradation of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) (Arabaci, 2011). Because of the high efficiency in degrading H_2O_2, catalase is widely used in the textile and paper industry for removing residual H_2O_2 after peroxide bleaching (Zeng et al., 2011). However, effective peroxide bleaching in these industries is usually achieved at a high-alkaline condition (pH > 9), that makes the exploitation of alkaline catalase to be necessary. At present, several alkaline catalases from biological strains have been successfully expressed and

* Corresponding author at: 32 XiQiDao, Tianjin Airport Economic Park, Tianjin 300308, China.
E-mail address: song_h@tib.cas.cn (H. Song).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biortech.2016.04.110
0960-8524/

characterized (Deng et al., 2008; Muster et al., 2015). Even so, such problems on industrial scale as low productivity, high production costs and complex fermentation bioprocess still prevent the industrial production of alkaline catalase.

The outstanding alkali resistance of alkaline catalases from *Bacillus* sp. strains indicated their great potential for application in textile industry. The alkaline catalases from different *Bacillus* sp. strains used to be successfully expressed in *Bacillus subtilis* WSHDZ-01, *B. subtilis* WB600 and *Escherichia Coli* BL21, achieving extracellular catalase production of 39,117 U/mL, 8449 U/mL and 28,990 U/mL respectively (Xu et al., 2014; Yao et al., 2009). Although greatly improved from the original level, the production still needs to be increased for industrial production. In this work, an alkaline catalase gene (*katA*) was firstly cloned from *B. subtilis* 168 and then was transformed into *E. coli* BL21 (DE3) for expression. Productivity and extracellular ratio of the recombinant catalase (KatA) was improved by optimizing the induction and cultivation conditions. The productivity of recombinant KatA was interestingly found to be enhanced by appropriate ethanol stress. And a moderate supplementation of Triton X-100 at the late phase of fermentation could effectively improve the extracellular ratio of recombinant KatA. Finally, the activity of total soluble recombinant catalase achieved 78,762 U/mL with the extracellular ratio of 92.5% at 48 h of fermentation in a 10 L fermentor. To the best of our knowledge, it is the highest productive level of alkaline catalase in recombinant *E. coli* so far. Furthermore, the efficiency of the recombinant catalase on elimination of hydrogen peroxide after cotton bleaching was also evaluated.

2. Materials and methods

2.1. Strains and plasmids

The *katA* gene was amplified by PCR from the genomic DNA of *B. subtilis 168*. The primers (forward: 5′-CGCCATGGCCATGAGTTCAAA TAAACT-3′; reverse: 5′-GCCTCGAGAGAATCTTTTTTAATCGGCAAT-3′) were designed according to the nucleotide sequence of *katA* from *B. subtilis*168 and contained restriction sites *Nco* I and *Xho* I, respectively. The amplified *katA* fragment was digested with restriction enzymes and was ligated into the pET26b (+) vector to construct the recombinant expression plasmid pET26b-*katA*. Then the pET26b-*katA* plasmid was transformed into *E. coli* BL21 (DE3) for expression of the recombinant alkaline catalase (KatA).

2.2. Media and feeding solution

Luria–Bertani (LB) medium was used in seed cultivation, which contained 10.0 g L^{-1} tryptone, 5.0 g L^{-1} yeast extract and 10.0 g L^{-1} NaCl. For shake-flask cultivation, terrific broth (TB) medium was used. TB medium consisted of 5.0 g L^{-1} glycerol, 12.0 g L^{-1} tryptone, 24.0 g L^{-1} yeast extract, 2.32 g L^{-1} KH_2PO_4 and 16.3 g L^{-1} K_2HPO_4. The synthetic medium used for batch fermentation in a 10 L fermentor contained 10.0 g L^{-1} glycerol, 1.7 g L^{-1} $MgSO_4{\cdot}7H_2O$, 1.0 g L^{-1} ammonium citrate, 2.0 g L^{-1} NaCl, 4.0 g L^{-1} $(NH_4)_2HPO_4$, 1.0 g L^{-1} $(NH_4)_2SO_4$, 6.0 g L^{-1} KH_2PO_4, 3.0 g L^{-1} $K_2HPO_4{\cdot}H_2O$ and 10.0 mL L^{-1} trace metal solution. The trace mental solution contained 5.0 g L^{-1} $CaCl_2$, 1.3 g L^{-1} sodium acetate, 0.25 g L^{-1} $CoCl_2$, 1.25 g L^{-1} EDTA, 1.5 g L^{-1} $MnCl_2$, 0.18 g L^{-1} $ZnSO_4$, 3.0 g L^{-1} $CuSO_4$, 10.0 g L^{-1} $FeSO_4$, 0.3 g L^{-1} $Na_2B_4O_7{\cdot}10H_2O$ and 20.0 mL L^{-1} 85% phosphoric acid. The feeding medium used for fed-batch fermentation was consist of 30.0 g L^{-1} glycerol, 30.0 g L^{-1} tryptone and 30.0 g L^{-1} yeast extract. All the medium for the recombinant strain cultivation were supplemented with 50 μg mL^{-1} kanamycin.

2.3. Cultivation conditions

2.3.1. Shake flask

For seed cultivation, 20 μL of glycerol stock strain was inoculated into 20 mL LB medium supplemented with 50 mg L^{-1} kanamycin in a 100 mL shake flask. The seed culture was cultivated at 37 °C in a rotary shaker at 220 rpm for 12 h. Then 500 μL seed culture was inoculated at 25 mL TB medium supplemented with 50 mg L^{-1} kanamycin in a 100 mL shake flask. The resulting culture was cultivated at 37 °C for 2 h in a rotary shaker at 220 rpm, when its optical density at 600 nm reached 0.6–0.8, isopropyl-β-D-thiogalactoside (IPTG) and ethanol were added to induce expression of the recombinant catalase. After that, the culture temperature was reduced to 27 °C and at 220 rpm for another 24 h. To release target protein from the cytoplasm, surfactant was added after 4–6 h of induction.

2.3.2. Fermentor

For primary seed cultivation, 20 μL of glycerol stock strain was inoculated into 20 mL LB medium containing 50 mg L^{-1} kanamycin in a 100 mL shake flask and cultivated at 37 °C in a rotary shaker at 220 rpm for 12 h. In the secondary seed cultivation, a 1.5% (v/v) primary seed culture was inoculated into 200 mL of the same medium in a 1 L shake flask and grown under the same condition. 4 L of synthetic medium supplemented with 50 mg L^{-1} kanamycin was prepared in a 10 L fermentor. The fermentation initiated with the inoculation of 200 mL secondary seed culture into the synthetic medium. Then a batch fermentation was conducted at 37 °C for 8–9 h, during which the dissolving oxygen (Do) and pH were maintained at 20% and 7 respectively. When the initial glycerol was exhausted, the phase of fed-batch fermentation was started. The flowing rate of fed-batch medium was put in cascade with Do level to kept oxygen concentration lower than 30%. Meanwhile, IPTG and ethanol were added into the medium to induce protein expression and cultivation temperature was adjusted to 27 °C. After 16 h of induction, surfactant was added to release target protein from the cytoplasm. In the entire fermentation process, pH was maintained at 7 by automatic addition of ammonia solution (25%). Oxygen concentration was kept at 30% by controlling the impeller speed (300–650 rpm) and air flow rate (0.167–0.833 vvm).

2.4. Preparation of KatA and SDS–PAGE

The culture was centrifuged at 13,800*g* for 8 min. Culture supernatants that comprised the extracellular soluble protein fraction were separated and harvested to detect extracellular catalase. For the pellet, cell disruption was performed to release the intracellular components. It was washed twice and resuspended by NaH_2PO_4–Na_2HPO_4 buffer solution (pH 7.0), then treated by ultrasonication at 50 Hz for 5 min in ice water. The disrupted mixture was centrifuged at 13,800*g* for 8 min. The supernatants were the intracellular soluble protein fraction and the disrupted pellet was the intracellular denatured protein fraction as inclusion body. All the samples were mixed with equal volume of SDS–PAGE loading buffer and heated at 100 °C for 10 min before electrophoresis. Polyacrylamide gel used in this work consisted of an upper stacking gel (5%, pH 6.8) and a lower separating gel (10%, pH 8.8). 20 μL of protein marker and all samples were injected into the wells of the gel respectively. Electrophoresis was performed in Tris–glycine buffer at 50 mA for 3 h. Then the gel was stained by coomassie brilliant blue R-250 for 4 h and destained by a destaining solution (80% acetic acid, 20% methanol) for 6–8 h.

2.5. Enzyme assay

H_2O_2 has an absorption peak at 240 nm, its decomposition caused by catalysis of catalase leads to decrease in absorbance at