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OPTIMIZATION OF THE *TRICHODERMA HARZIANUM* CuZn SUPEROXIDE DISMUTASE GENE EXPRESSION IN *E. COLI*

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ABSTRACT

A study of the optimization of CuZn superoxide dismutase gene expression in *E. coli* L21/pET28-SOD was undertaken. The SOD gene was directionally cloned into expression vector pET28.The recombinant plasmid was transformed into *E. coli* BL21 and induced by IPTG to express SOD protein. The four effective factors, including the initial concentration of BL21/pET28-SOD, concentration of IPTG, incubation temperature and induction time, were optimized during the induction of target protein. To detect the expression efficiency under different induction conditions, the total protein in the samples were subjected to SDS-PAGE. The SOD protein was successfully expressed in *E. coli* BL21/pET28-SOD. SOD expression was optimal at the following that *E. coli* BL21/pET28-SOD at OD_{600nm} =0.6, IPTG 0.125 mmol/L, incubation at 37℃ and incubation for four hours.

KEYWORDS: SOD protein, expression, optimization

1. INTRODUCTION

Superoxide dismutases (SODs) are an ubiquitous family of enzymes that can efficiently catalyze the dismutation of superoxide anions. They are the first and most important line of antioxidant enzyme defense systems against reactive oxygen species (ROS) [1]. The true catalytic function of SOD was discovered by McCord and Fridovich [2]. Thus far, four types of SOD have been identified and differed by the metallic ion present at the active site: copper and zinc (CuZnSOD), manganese (MnSOD), iron (FeSOD) or nickel (NiSOD) [3]. SOD is found in virtually all oxygen-consuming organisms. Deletions or mutations of SOD genes can lead to severe biological disorders [4]. Most oxygen-consuming organisms protect themselves from superoxide anions by expressing superoxide dismutases. SODs efficiently dismutate the superoxide anions into oxygen and hydrogen peroxide. They thereby prevent the superoxide anion from initiating a radical chain reaction that could result in other ROS provoking damage to nucleic acids, proteins, and membrane lipids [5].

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Expression of recombinant human CuZnSOD in *E. coli* was first described in 1985, high-level expression in *E. coli* in 1986, over-expression in yeast in 1987 and baculovirus systems were reported in 1995 [6-9]. In recent years, there have been a significant number of extensive SOD studies in a variety of other plant species, in particular, Arabidopsis, tobacco, tomato, and rice [10-14]. Species of *Trichoderma* are commercially applied as biological control agents against plant fungal pathogens based on different mechanisms, such as the production of antifungal metabolites, competition for space and nutrients with mycoparasitism. In our previous experiment a directional cDNA library has been constructed from mycelium of *T. harziamum* and 3298 clones have been selected randomly, subjected to single-pass sequencing from the 5' end of the vector and identified by sequence similarity searches against gene sequences in international databases. Of the 3298 mycelium clones, 2174 exhibit similarity to known genes and 451 to known ESTs, while 673 represent novel gene sequences [15]. The silico cloning of full-length cDNA of CuZn SOD is acquired from the cDNA library of T. harziamum. In this paper, we have expressed the CuZn SOD protein and studied the optimization of the expression conditions in *E.coli*.

2. MATERIALS AND METHODS

2.1 Materials

E. coli BL21/pET28-SOD was constructed by YANG Li-Ming, PHD, Department of Life Science and Engineering, Harbin Institute of Technology. Vector pET28a and *E. coli* BL21 were stored at department of Life Science and Engineering, Harbin Institute of Technology.

2.2 Induced expression using different incubation periods

2 ml of overnight culture of *E. coli* BL21 harboring the pET28-SOD recombinant plasmid was inoculated into 50ml LB containing 50μg/ml kanamycin. The cultures were incubated at 37℃ by shaking for 2 to 3 h, until the OD_{600nm} was about 0.5. After taking 1ml sample as uninduced control, the cultures were incubated at 37℃ with sampling of 1ml after 1.0h, 2.0h, 3.0h, 4.0h and 5.0h induction. The cells were collected by centrifugation and stored at -20℃. The culture samples obtained at different induction times were lysed in 2×SDS sample buffer, and SDS-PAGE was employed to detect the SOD protein.

2.3 Induced expression using different IPTG concentrations

2 ml of overnight culture of *E. coli* BL21 harboring the pET28-SOD recombinant plasmid was inoculated into 50ml LB containing 50μg/ml kanamyacin. The culture containing IPTG at final concentrations of 0.125mM, 0.25mM, 0.5 mM, 1.0mM, 2.0 mM and 4.0 mM were prepared. After incubation at 37℃ for 4 h, 1ml samples from each of the cultures were collected and dealt with as described previously.

2.4 Induced expression under different initial concentrations of BL21/pET28-SOD

2 ml of overnight culture of *E. coli* BL21 harboring the pET28-SOD recombinant plasmid was inoculated into 50 ml LB containing 50μg/ml kanamyacin. The culture was incubated at 37℃ by shaking for 2 to 3h. After taking 1ml sample as uninduced control, This part should be rewritten. Incubation of culture at 37℃ by shaking for 2-3 hrs before addition of IPTG will result in OD_{600nm} of ~ 0.5 according to data presented here. The culture was incubated at 37℃ for 4 h with sampling of 1ml after OD_{600nm} 0.148，0.312，0.582，0.810，1.040 induction. The samples were dealt with as described.

2.5 Induced expression under different incubation temperatures

2 ml of overnight culture of *E. coli* BL 21 harboring the pET28-SOD recombinant plasmid was inoculated into 50 ml LB containing 50 µg/ml kanamyani. The culture was incubated at 37℃ by shaking for 2 to 3 h until the OD_{600nm} was about after taking 1ml sample as uninduced control, IPTG was added to the culture to a final concentration of 0.1mmol/L. The cultures were incubated at 30℃, 34℃, 37℃ and 39℃ for 4 h with sampling of 1ml. The samples were deat with as discussed above.

3. RESULTS AND DISCUSSION

3.1 Induced expression at different incubation periods

A specific band of protein at the position about 15.7kD was detected by SDS-PAGE (Figure1), which was similar to that predicted. The amount of expression was significantly increased with extension of induction time, and it reached the peak after 4h induction with the target protein.

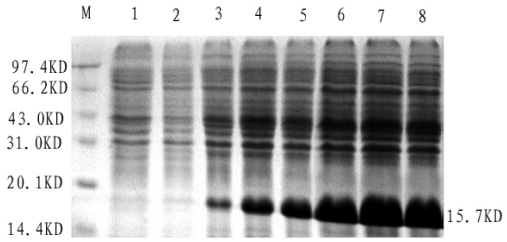

Figure 1 Analysis of induced expression at different incubation periods M: Protein Markers; Lane1: E.coli BL21; Lane2: pET/BL21; Lane3: pET-SOD/BL21 [Uninduced] Lane 4~9: pET-SOD/BL21 induced for 1 h, 2 h, 3 h, 4 h, 5 h respectively

3.2 Induced expression at different levels of IPTG

A specific band of protein at the position about 15.7 kD was detected by SDS-PAGE (Figure 2), which was similar to that predicted. It reached the peak at IPTG concentration of 0.125mM.

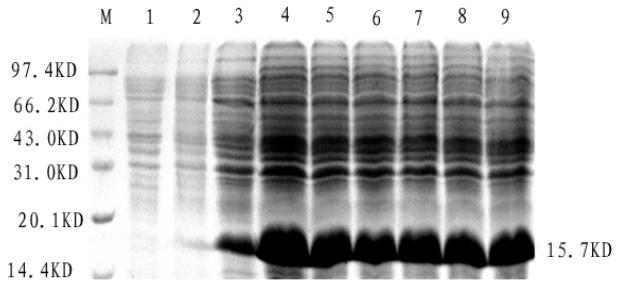

Figure 2 Analysis of induced expression at different levels of IPTG concentrations. M: Protein Markers; Lane1: E.coli BL21; Lane2: pET/BL21; Lane3: pET-SOD/BL21

[Uninduced] Lane 4~9: pET-SOD/BL21 induced at different IPTG concentration [0.125mM, 0.25mM, 0.5 mM, 1.0 mM, 2.0 mM, 4 mM]

3.3 Induced expression at different initial concentrations of BL 21/pET28-SOD

A specific band of protein at the position about 15.7 kD was detected by SDS-PAGE (Figure 3), which was similar to that predicted. It reached the peak at initial concentration OD_{600nm} 0.582.

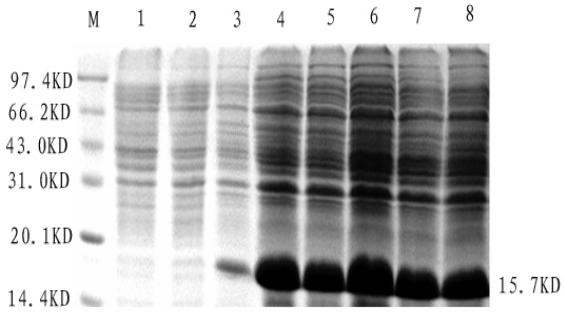

Figure 3 Analysis of induced expression at different initial concentrations of BL21/pET-SOD M: Protein Markers; Lane1: *E.coli* BL21; Lane2: pET/BL21; Lane3: pET-SOD/BL21 [Uninduced] Lane 4~8: pET-SOD/BL21 at different initial concentrations [incubation subscript OD600＝0.148，0.312，0.582，0.810，1.040]

3.4 Induced expression at different temperatures

A specific band of protein at the position about 15.7kD was detected by SDS-PAGE (Figure 4), which was similar to that predicted. It reached the peak at 37℃ induction with the target protein.

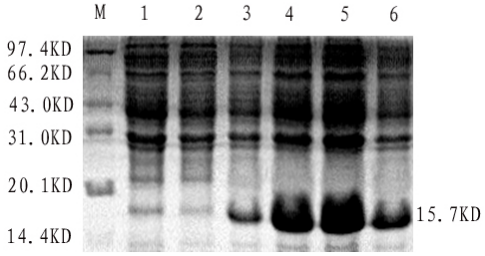

Figure 4 Analysis of induce expression of different temperature M: Protein Markers; Lane1: E. coli BL21; Lane2: pET/BL21; Lane 3~6: pET-SOD/BL21 induced at 30, 34, 37, 39℃respectively

(The 6×His affinity tag facilitated binding to Ni-NTA. It is poorly immunogenic and the tag is small, uncharged, and therefore does not generally affect secretion, compartmentalization, or folding of the fusion protein within the cells [16]. In most cases, the 6×His tag does not interfere with the struction or function of the purified protein.)

The further gain of knowledge about the mechanisms of cell and tissue-specific regulation of SOD gene expression and their signal transduction pathways may lead to the design

of new drugs and strategies directed at regulating levels of these enzymes in particular tissues, cell types, and compartments without affecting other cells [1].

We have studied the optimal expression condition of SOD protein in *E.coli*. The optimization conditions were OD_{600nm} 0.6, IPTG 0.125mmol/L, incubation at 37℃ and incubation for 4 hours. This provided the foundation for studying the molecular mechanism of *Trichoderma harzianum* in future research.

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