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Prokaryotic Expression of Recombinant Fusion Gene CAT-SYI from Streptococcus Mutans

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ABSTRACT

Objective: To express and purify the recombinant fusion protein CAT SYI of Streptococcus mutans, and to provide basic materials for subsequent immunogenicity research and the development of anti caries vaccines. Methods: The recombinant plasmid pET32a CAT SYI constructed previously in our laboratory was transformed into Escherichia coli DH5α for amplification. After PCR verification, the correctly identified plasmid was transformed into the expression strain BL21. The expression of the recombinant fusion protein was induced by IPTG, and the induction conditions including IPTG concentration (0.25-1.5mM), induction time (0-10h) and induction temperature (26℃,37℃) were optimized. The bacterial cells were disrupted by ultrasonication, and the recombinant protein was purified and refolded by urea gradient dialysis. The expression and purification efficiency were analyzed by SDS PAGE. Results: PCR identification confirmed that the recombinant plasmid pET32a CAT SYI was successfully constructed, with an amplified fragment of approximately 750bp. SDS PAGE analysis showed that the recombinant fusion protein was successfully expressed with a molecular weight of about 50kDa, which was consistent with the expected value. The optimal induction conditions were determined as follows: final IPTG concentration of 1.25mM, induction time of 6 h, and induction temperature of 37℃. Soluble target protein was obtained after dialysis and refolding of the purified recombinant protein. Conclusion: The high efficiency expression of the recombinant fusion protein CAT SYI from Streptococcus mutans in Escherichia coli was successfully achieved, and the corresponding induction optimization and purification methods were established. These results lay an experimental foundation for the subsequent evaluation of immune protective efficacy and the research and development of anti caries vaccines.

KEYWORDS

Fusion gene CAT-SYI; Protein expression; Induction

1. INTRODUCTION

Dental caries is one of the most prevalent chronic infectious diseases worldwide and severely compromises human oral health and quality of life. The oral cavity harbors one of the most diverse microbial communities in the human body, and disruption of the oral ecological balance can result in oral diseases including dental caries [1]. According to statistics from the World Health Organization (WHO), approximately 2.5 billion people globally suffer from dental caries in permanent or primary teeth, and untreated dental caries in primary teeth alone affects 573 million children worldwide [2]. Dental caries has emerged as a major global public health problem.

Streptococcus mutans (S. mutans) is currently recognized as the primary cariogenic bacterium. It is a facultative anaerobic Gram-positive coccus with eight serotypes (a-h). The cell wall antigenic components differ among distinct serotypes, among which serotype c shows the highest detection rate in humans [3]. The cariogenic virulence of S. mutans is largely associated with its interactions with other oral microorganisms, some of which can potentiate the functions of S. mutans virulence factors [4]. Studies have shown that Candida albicans interacts with S. mutans to strengthen the biofilm matrix. C. albicans enhances the growth and virulence of S. mutans via the ergosterol pathway, thereby promoting the pathogenicity of cariogenic microbes. Callahan Katrak et al. [5] reported that catalase derived from C. albicans protects S. mutans from hydrogen peroxide-mediated damage.

In recent years, anti-caries strategies have become a research hotspot, including novel approaches such as immune anti-caries therapy, antimicrobial peptides, probiotics, nanoparticles, and photodynamic therapy [6]. Immune anti-caries therapy is particularly noteworthy, which elicits host immune responses through artificial antigen administration, with the goals of enhancing immunogenicity and simplifying vaccine preparation. The protein encoded by the CAT gene displays favorable immunoreactivity, while the SYI gene fragment is potentially related to the function of glucan-binding proteins. Fusion of these two genes is expected to induce more comprehensive immune protection. In this study, the recombinant plasmid pET32a-CAT-SYI constructed previously in our laboratory was transformed into Escherichia coli BL21. The recombinant fusion protein was expressed under IPTG induction, and the induction conditions were optimized for protein purification. This work aims to provide fundamental materials for subsequent immunogenicity research and the development of anti-caries vaccines.

2. MATERIALS AND METHODS

2.1. Main Materials and Reagents

Prestained protein Marker, Thermo Fisher Scientific; Urea, Kelong Chemical Reagent Factory; Glycine, Biofroxx; Dialysis tubing, Biosharp; Arginine, Biofroxx; Ampicillin sodium, Solarbio; SDS, Amresco; Plasmid DNA Mini Extraction Kit, Sangon Biotech.

2.2. Main Instruments and Equipmen

Autoclave, Jiangyin Binjiang Medical Equipment Co., Ltd.; PCR thermal cycler, G-STORM, UK; Biochemical incubator, Shaoguan Taihong Medical Equipment Co., Ltd.; Protein electrophoresis system, BIO-RAD; Gel imaging analysis system, Tanon; Nucleic acid electrophoresis system, Puyang Scientific Research Institute; Ultrasonic cell disruptor, Ningbo Xinzhi Biotechnology Co., Ltd.; Constant temperature shaking incubator, Wuhan Ruihua Instrument & Equipment Co., Ltd.; Ice maker, Changshu Xueke Electric Appliance Co., Ltd.; Ultra-low temperature freezer, Froilabo BMS, France.

2.3. Experimental Methods

2.3.1. Expression of Recombinant Fusion Protein

DH5α competent cells were prepared by the calcium chloride method. A 100 µL aliquot of competent cells was mixed with 2-5 µL of pET32a-CAT-SYI, incubated on ice for 30 min, heat-shocked in a 42 ℃ water bath, and then placed on ice for another 2-3 min. After adding 1 mL of LB medium, the cells were incubated at 37 ℃ for 0.5 h. A 100 µL volume of the bacterial suspension was spread onto an LA plate supplemented with 10 µL Amp (100 mg/mL), placed upright for 15 min, and then incubated inverted at 37 ℃ for 18-24 h.

A single colony was picked from the DH5α plate transformed with the recombinant plasmid CAT-SYI and inoculated into 50 mL of LB liquid medium containing Amp (final concentration 50 µg/mL), followed by shaking incubation at 37 ℃ and 220 rpm for 12-14 h. The bacterial culture was collected, and plasmid extraction was performed using a Sangon plasmid mini extraction kit.

PCR was performed as follows: pre-denaturation at 94 °C for 1 min; touch-down PCR procedure: denaturation at 94 °C for 1 min, annealing starting from 68 °C and decreasing to 64 °C for 40 s, extension at 72 °C for 1 min, with 2 cycles per temperature step; followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 62 °C for 40 s, and extension at 72 °C for 1 min; final extension at 72 °C for 5 min. PCR products were detected by 1% agarose gel electrophoresis.

2.3.2. Expression of Recombinant Fusion Protein Induced by IPTG

A single colony was inoculated into 10 mL of LB liquid medium supplemented with 5 μL Amp (100mg/mL) to a final concentration of 50 μg/mL, and cultured at 37 °C with shaking at 220 rpm until the OD_{600} value reached 0.6-0.8. After sample collection, 10 μL of 1 M IPTG was added to a final concentration of 1.0 mM, followed by shaking incubation at 37 °C and 220 rpm for 6 h, and 1 mL of the culture was sampled. Samples collected before and after induction were analyzed by SDS-PAGE.

Single colonies on the plate were picked and subjected to two rounds of activation, then aliquoted into five 50 mL centrifuge tubes with 10 mL bacterial culture per tube. IPTG (1 M) was added to the five tubes at 2.5 μL, 7.5 μL, 10 μL, 12.5 μL, and 15 μL, corresponding to final concentrations of 0.25 mM, 0.75 mM, 1.0 mM, 1.25 mM, and 1.5 mM, respectively. The cultures were incubated at 37 °C with shaking at 200 rpm for 6-8 h. Samples before and after induction were detected by SDS-PAGE to determine the optimal IPTG concentration for induction.

Based on the optimal IPTG concentration determined above, a time gradient of induction expression (0, 4, 6, 8, and 10 h) was set. Subsequently, induction expression was compared at 26 °C and 37 °C to determine the appropriate induction temperature. Samples were collected before and after induction and analyzed by SDS-PAGE.

2.3.3. Purification of Recombinant Protein

Under optimal expression conditions, the harvested cells were washed twice with sterile PBS. The cells were resuspended in 20 mL sterile PBS per gram of cell pellet, and lysozyme was added to a final concentration of 0.1 mg/mL. The mixture was incubated at 4 °C for 1-2 h until viscous, followed by ultrasonic disruption at 200 W (6 s sonication, 5 s interval, for a total of 40 min). After centrifugation at 10000 r/min and 4 °C for 10 min, the pellet was collected, washed and ground twice with 1 M urea-PBS buffer containing 0.1% Triton. After centrifugation, the pellet was washed and ground twice with 2 M urea-PBS buffer, and the inclusion bodies were harvested by centrifugation and weighed.

The inclusion bodies were resuspended in 40 mL PBS containing 8 mol/L urea per gram for 20 min, then centrifuged at 10000 r/min and 4 °C for 10 min. The supernatant was transferred into dialysis tubing for gradient refolding. After completion of dialysis, the sample was centrifuged at 10000 r/min and 4 °C for 10 min. The supernatant was collected, and the result of protein purification was verified by SDS-PAGE.

3. RESULTS AND ANALYSIS

3.1. PCR Identification of the Recombinant Plasmid

As shown in Figure 1, the recombinant plasmid extracted from DH5α cells using a plasmid mini-extraction kit was analyzed by 1% agarose gel electrophoresis after touchdown PCR. A distinct nucleic acid band at approximately 750 bp was observed, indicating that the recombinant plasmid was correctly constructed.

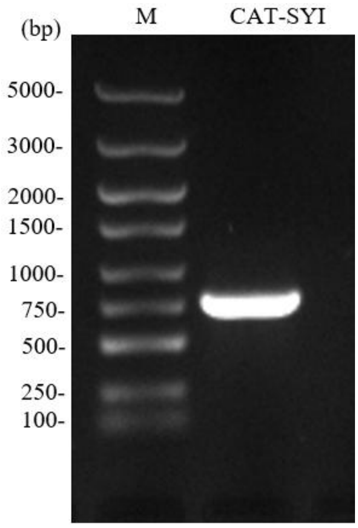

Figure 1. Agarose gel electrophoresis of PCR amplification products

3.2. IPTG-Induced Expression of Recombinant Fusion Protein

Escherichia coli BL21 transformed with pET32a-CAT-SYI was induced with 1.0 mM IPTG. A distinct target band around 50 kDa was detected after induction (Figure 2), which was consistent with the expected molecular weight. SDS-PAGE analysis demonstrated that the recombinant fusion protein was successfully expressed.

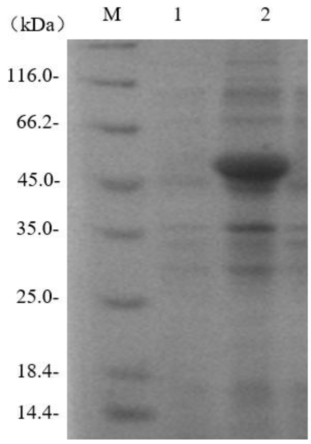

Figure 2. Induced Expression of Recombinant Fusion Protein

3.3. Comparison of Induction Results Under Different IPTG Concentrations, Times and Temperatures

To obtain the optimal expression conditions for the recombinant fusion protein CAT-SYI, the IPTG concentration, induction time and induction temperature were optimized in this study. The SDS-PAGE results are shown in Figure 3 to Figure 5.

In the IPTG concentration optimization, five final concentration gradients (0.25, 0.75, 1.0, 1.25 and 1.5 mM) were set, and induction was performed at 37 °C for 6 h. The results (Figure 3) showed that target protein bands were observed at all concentrations, and the highest expression level was found in Lane 8 (1.25 mM), which was determined as the optimal induction concentration.

In the induction time optimization, IPTG concentration was fixed at 1.25 mM, and induction was carried out at 37 °C for 0, 4, 6, 8 and 10 h. The results (Figure 4) showed that the protein expression level reached the maximum at 6 h of induction (Lane 4), and then decreased slightly with prolonged induction. Therefore, 6 h was determined as the optimal induction time.

In the induction temperature optimization, induction was performed with 1.25 mM IPTG at 26 °C and 37 °C for 6 h, respectively. The results (Figure 5) showed that the protein expression level in the 37 °C group (Lane 3) was significantly higher than that in the 26 °C group (Lane 2), indicating that 37 °C was more favorable for the expression of the recombinant protein.

In conclusion, the optimal induction conditions for recombinant fusion protein CAT-SYI were determined as follows: 1.25 mM IPTG, induction for 6 h at 37 °C. Large-scale expression of the recombinant protein in subsequent experiments was performed under these optimized conditions.

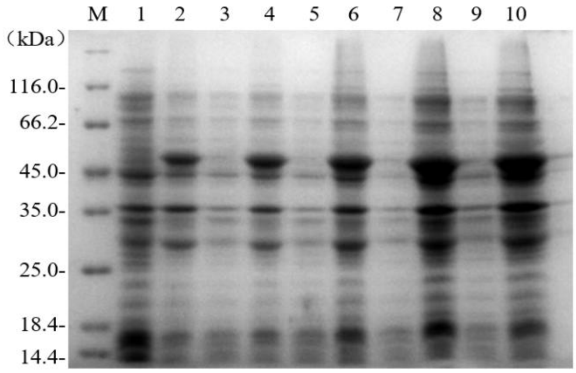

Figure 3. SDS-PAGE of Induced Expression with Different IPTG Concentrations

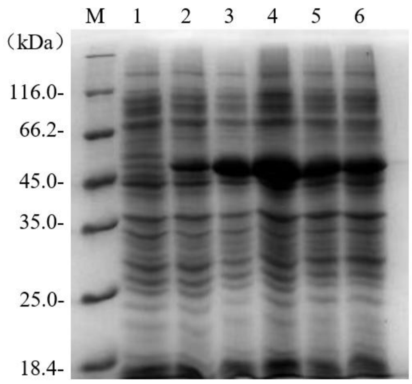

Figure 4. SDS-PAGE Analysis of Induced Expression at Different Induction Times

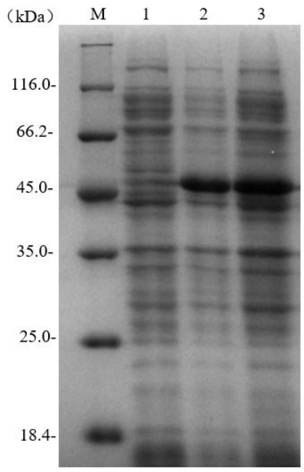

Figure 5. SDS-PAGE Analysis of Induced Expression at Different Induction Temperatures

3.4. Purification Result of the Recombinant Fusion Protein

The washed inclusion bodies were dissolved in 8 M urea. The denatured and solubilized crude inclusion body protein was refolded by gradient dialysis using a urea-PBS buffer system. Samples of the refolded protein solution were analyzed by SDS-PAGE. As shown in Figure 6, a clear target band around 50 kDa was observed with low levels of contaminating proteins, indicating high purity of the recombinant protein, which was consistent with the expected result.

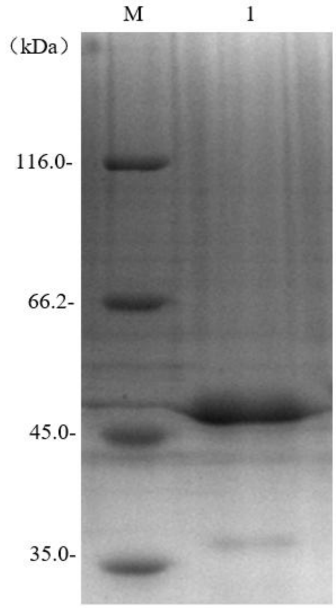

Figure 6. Electrophoretogram of Purified Recombinant Protein

4. DISCUSSION

Dental caries, one of the most common chronic infectious diseases worldwide, shows an especially high prevalence in economically disadvantaged populations [7]. It can cause a series of secondary oral diseases, including pulpitis, periodontitis and apical periodontitis, and may even trigger life-threatening diseases such as infective endocarditis and ulcerative colitis, which severely affect human daily life [8]. Therefore, the research on the prevention and treatment of dental caries has long been a hotspot in stomatology. Streptococcus mutans is the major cariogenic pathogen [9]. Its cariogenic mechanisms are mainly related to acid production and acid tolerance, as well as polysaccharide synthesis, bacterial adhesion, colonization and cariogenic biofilm formation [10]. Its surface protein antigen (SA) and glucosyltransferase (GTF) play key roles in bacterial adhesion, biofilm formation and cariogenic processes. Immune intervention strategies targeting these critical virulence factors, especially the development of genetically engineered vaccines, have become an important direction in anti-caries research. In this study, the prokaryotic expression of the S. mutans recombinant fusion protein CAT-SYI was successfully achieved in Escherichia coli, and the induction conditions were optimized and the protein was purified, laying a foundation for subsequent immunogenicity research.

The pET32a expression system adopted in this study has a strong promoter and an efficient translation initiation signal, which can realize high-level expression of exogenous genes. PCR identification showed that the recombinant plasmid pET32a-CAT-SYI was successfully constructed, and the size of the amplified fragment was about 750 bp, which was consistent with the expectation. SDS-PAGE analysis indicated that the recombinant fusion protein was successfully expressed after IPTG induction, with a molecular weight of approximately 50 kDa, which was consistent with the theoretical molecular weight of the CAT-SYI fusion protein, proving that the fusion gene was correctly translated in E. coli BL21.

Optimization of induction conditions is the key step to improve the yield of recombinant proteins. In this study, IPTG concentration, induction time and temperature were systematically optimized. The results showed that the highest protein expression level was obtained at a final IPTG concentration of 1.25 mM, which was higher than the conventional 1.0 mM. This phenomenon may be related to the structural characteristics of the target protein or the metabolic state of the host strain. A high expression level could be achieved after 6h of induction, and prolonging the induction time to 8-10 h did not significantly increase the yield, but might lead to protein degradation due to bacterial aging. The temperature optimization revealed that induction at 37 °C was better than that at 26 °C, suggesting that a relatively high temperature was more favorable for the expression of this fusion protein, which was consistent with the characteristics of most prokaryotic expression systems.

In summary, the prokaryotic expression of the S. mutans recombinant fusion protein CAT-SYI was successfully realized, the optimal induction conditions were determined, and a corresponding purification method was established in this study. Further research will be carried out to evaluate the immunogenicity and anti-caries effect of this recombinant protein, so as to provide experimental evidence for its feasibility as a candidate anti-caries vaccine.

5. CONCLUSION

In this study, the recombinant plasmid pET32a-CAT-SYI constructed in our laboratory was successfully transformed into Escherichia coli DH5α and BL21. Touchdown PCR verification confirmed the correct construction of the recombinant plasmid, and the size of the target fragment was approximately 750 bp, which was consistent with the expected result. The prokaryotic expression of the Streptococcus mutans recombinant fusion protein CAT-SYI in E. coli BL21 was achieved by IPTG induction. SDS-PAGE analysis showed that the molecular weight of the fusion protein was about 50 kDa, which was consistent with the theoretical value, verifying that the target gene was correctly translated in the host strain. Through systematic optimization of induction conditions, the optimal expression conditions for the fusion protein were determined as a final IPTG concentration of 1.25 mM and induction at 37 °C for 6 h, under which the protein expression level reached the peak. Meanwhile, the purified CAT-SYI recombinant fusion protein was successfully obtained.

This study successfully realized the high-efficiency prokaryotic expression and purification of the CAT-SYI fusion protein and clarified its optimal induction conditions. It provides key experimental materials and technical support for the subsequent immunogenicity detection and verification of the caries-preventive protective effect of the protein in vitro and in vivo, and also lays an important experimental foundation for the research and development of S. mutans genetically engineered caries-preventive vaccines. Further research will be conducted to explore the immunogenicity, immune protective efficacy and related mechanisms of the recombinant fusion protein, so as to provide scientific evidence for its development as a candidate caries-preventive vaccine.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Pan FENG: Writing-original draft, conceived the research, analyzed the data. Zaixin LI: Supervision designed and supervised the experimental work. Zhi ZHANG: Supervision, designed and supervised experimental work. Xiaohong JIAN: Supervision, designed and supervised experimental work.

DECLARATION OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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