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Construction of an Escherichia coli Cell Lysis System and Its Application in Mycotoxin-Degrading Enzyme Expression: Postprint

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Abstract

Objective: Secretory expression of recombinant proteins in Escherichia coli is limited by its secretion efficiency; to address this, an inducible lysis system for E. coli was designed and constructed to achieve rapid and efficient secretion of intracellular recombinant proteins. Methods: Utilizing the lytic capability of colicin E7 against cells, an E. coli cell lysis system co-expressing the target recombinant protein and E7 was constructed, enabling the target recombinant protein to be released into the culture medium upon E7 expression. Results: First, using red fluorescent protein (RFP) as a reporter gene, two expression cassettes for colicin E7 and RFP were constructed on the pET28a(+) vector. Through comparative analysis of protein expression performance between one-step IPTG induction and IPTG-arabinose stepwise induction systems, the stepwise induction system was found to more efficiently express and release target proteins into the medium. The zearalenone-degrading enzyme gene was expressed in the IPTG-arabinose stepwise induction lysis system, and the culture supernatant was found to contain good expression levels and high activity of the zearalenone-degrading enzyme, capable of degrading approximately 5.8 g of zearalenone toxin under reaction conditions of 37 °C for 30 min. Conclusion: An E. coli cell lysis system was successfully constructed utilizing colicin E7, and this system demonstrates applicability for the rapid release of intracellularly expressed foreign proteins.

Full Text

Preamble

Construction and Application of Cell Lysis Systems for Mycotoxin Degrading Enzyme Expression in *Escherichia coli*

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Abstract

Objective: Secretory expression of recombinant proteins in *Escherichia coli* is limited by low secretion efficiency. To address this, we designed and constructed an inducible cell lysis system in *E. coli* to achieve rapid and efficient release of intracellular recombinant proteins. **Methods:** Leveraging the cell lytic capability of colicin E7, we constructed *E. coli* cell lysis systems that co-express target recombinant proteins with E7, enabling release of the target proteins into the culture medium following E7 expression. **Results:** Using red fluorescent protein (RFP) as a reporter gene, we constructed two expression cassettes for colicin E7 and RFP on the pET28a(+) vector. Comparative analysis of IPTG single-induction and IPTG-arabinose two-step induction systems revealed that the two-step induction system achieved more efficient expression and release of target protein into the medium. When applied to express the zearalenone degrading enzyme gene, the two-step inducible lysis system yielded substantial expression and high activity in the culture supernatant, degrading approximately 5.8 g of zearalenone toxin within 30 minutes at 37°C. **Conclusion:** We successfully constructed an *E. coli* cell lysis system utilizing colicin E7, demonstrating its applicability for rapid release of intracellularly expressed foreign proteins.

Keywords: *E. coli*; cell lysis system; red fluorescent protein; zearalenone degrading enzyme

Introduction

Escherichia coli is a widely used microbial host for protein biosynthesis. Compared with other prokaryotic expression systems, *E. coli* offers clear genetic background, rapid reproduction, high protein expression levels, low cost, and broad applicability [?]. However, intracellular expression of recombinant proteins in *E. coli* increases cell disruption and separation costs. Current bacterial cell disruption methods include mechanical approaches such as high-pressure homogenization, glass bead grinding, and ultrasonication, as well as non-mechanical methods like detergent lysis, alkaline lysis, and enzymatic cell wall degradation [?]. Mechanical methods typically generate heat that can inactivate target proteins, while non-mechanical methods suffer from high costs.

To address challenges associated with intracellular expression and cell disruption, secretory expression strategies for recombinant proteins have attracted considerable attention. Gram-negative bacteria possess five natural secretion systems, with Type I and Type II being commonly used for heterologous protein secretion in *E. coli* [?]. The *E. coli* -hemolysin (HlyA) secretion system

represents the most typical Type I system [?, ?], but uncleaved signal sequences may affect protein activity, and recombinant protein yields are often low due to competitive protein expression. The Type II secretion pathway is a two-step process mediated by periplasmic transport, which is complex and suffers from low secretion efficiency [?].

Numerous signal peptides from *E. coli* and other sources have been successfully employed for recombinant protein secretion in *E. coli* [?, ?]. Native *E. coli* signal peptides are most commonly used; signal peptides from outer membrane proteins such as OmpA and OmpF can facilitate secretion of fusion-expressed recombinant proteins into the culture medium [?], while signal peptides from periplasmic proteins like alkaline phosphatase (PhoA) can direct recombinant proteins to the periplasm [?], from which they can be released into the extracellular medium by expressing bacteriocin release proteins (BRP) [?]. Signal peptides from other secreted proteins, such as pectate lyase (PelB) from *Erwinia carotovora* [?] and signal peptides from *Bacillus* [?], have also successfully secreted recombinant proteins in *E. coli.* However, signal peptide-mediated secretion suffers from low efficiency, poor universality, and limited applicability.

Constructing programmed cell lysis systems in *E. coli* provides a novel approach for secretory expression of recombinant proteins. This strategy eliminates the need for signal peptides; instead, lysis-promoting proteins are expressed to induce cell lysis and release recombinant proteins into the extracellular medium. Morita et al. [?] achieved -glucuronidase production through *E. coli* lysis by expressing T4 phage lysis proteins. Yang et al. [?] expressed temperature-sensitive T4 lysozyme in *E. coli* to release and recover -galactosidase and other proteins. Lo et al. [?] designed a synthetic genetic circuit enabling cell density-dependent auto-regulatory lysis for macromolecule release, integrating a carbon starvation promoter, quorum sensing regulatory unit, and colicin E7 to extract intracellular plasmid DNA. Colicin E7 first damages the inner membrane and subsequently activates outer membrane phospholipase A (OMPLA) to cause cell lysis [?, ?].

In this study, we constructed an *E. coli* cell lysis system that co-expresses target proteins with colicin E7, inducing E7 expression to initiate cell lysis and release recombinant proteins into the medium. We linked two expression cassettes for colicin E7 and red fluorescent protein (RFP) to the pET28a(+) vector, comparing an IPTG single-induction lysis system where the T7 promoter simultaneously controls both colicin E7 and target protein expression, with a two-step induction system where the arabinose promoter (araBAD) controls colicin E7 and the T7 promoter controls target protein expression. Results demonstrated that the two-step induction system achieved higher target protein expression levels. Based on these findings, we applied the two-step induction system to produce zearalenone degrading enzyme (ZENd), validating the system' s applicability by detecting both expression level and activity of the released protein.

Materials and Methods

1.1 Strains, Plasmids, and Primers

The strains and plasmids used in this study are listed in Table 1 . Primers were synthesized by Genewiz Biotech (Beijing) Co., Ltd., with sequences provided in Table 2 .

Table 1. Strains and plasmids used in this study

Strains and plasmids	Characteristics	Source
E. coli DH5	F ; 80lacZΔM15; Δ(lacZYA-argF)U169; deoR; recA1; endA1; hsdR17(r , m); phoA; supE44; ; thi-1; gyrA96; relA1	Lab collection
E. coli BL21(DE3)	F ; ompT; hsdSB(rB , mB); gal; dcm(DE3)	Lab collection
pKD46	Ampr, -Red recombinase under araBAD promoter, temperature-conditional replicon	Lab collection
pET28a(+)	Kanr, T7lac promoter, His-Tag (N, C) and T7-Tag (I)	Lab collection
pET28a(+)-E7	pET28a(+) containing E7 gene	This study
pET28a(+)-Ara-E7	colicin E7 under the control of araBAD promoter in pET28a(+)-E7	This study
pET28a(+)-T7E7	pET28a(+) containing a cassette of colicin E7 controlled by T7 promoter	This study
pET28a(+)-T7E7-T7rfp	pET28a(+)-T7E7 containing a cassette of rfp controlled by T7 promoter	This study
pET28a(+)-AraE7	pET28a(+) containing a cassette of colicin E7 controlled by araBAD promoter	This study

Strains and plasmids	Characteristics	Source
pET28a(+)-AraE7-T7rfp	pET28a(+)-AraE7 containing a cassette of rfp controlled by T7 promoter	This study
pET28a(+)-AraE7-T7ZENd	pET28a(+)-AraE7 containing a cassette of ZENd controlled by T7 promoter	This study

Table 2. Primers used in this study

Primers	Sequences (5' →3')	Restriction sites	Target genes
E7-F	5' -CATGCCATGGATGAAAAAAATAACAGG-3'	Nco I	Colicin E7
E7-R	5' -CCGCTCGAGTTACTGCGTTTCCACTCC-3'	Xho I	Colicin E7
PBAD-F	5' -CACTGATCCGCATGCTTATGAC-3'	Sph I	ParaBAD
PBAD-R	5' -TCTAGATCTAGATTCCCAAAAAAACGGGTATGGA-3'	Xba I	ParaBAD
rfp-F	5' -CCGGAATTCATGGCTTCCTCCGAAGACGTTATC-3'	EcoR I	RFP
rfp-R	5' -ACGCGTCGACTTAAGCACCGGTGGAGTGACGACC-3'	Sal I	RFP
ZENd-F	5' -CCGGAATTCATGCGTATCCGTAGCACCATTAG-3'	EcoR I	ZENd
ZENd-R	5' -ACGCGTCGACTTACAGATATTTCTGGGTAAATTC-3'	Sal I	ZENd

Underline sequences indicate restriction enzyme cleavage sites.

1.2 Enzymes and Reagents

Taq polymerase, PrimeSTAR HS DNA polymerase, and DNA Ligation Kit were purchased from Takara Bio (Beijing) Co., Ltd. Plasmid extraction kits, DNA gel recovery kits, and PCR purification kits were obtained from Axygen Biotech (Hangzhou) Co., Ltd. Fast restriction enzymes were from Thermo Fisher Scientific (China) Co., Ltd. Isopropyl -D-thiogalactoside (IPTG) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Tryptone and yeast extract were from OXOID. All other routine reagents were analytical grade from domestic sources.

1.3 Culture Media

LB medium composition: 10 g/L NaCl, 10 g/L tryptone, 5 g/L yeast extract, used for activation and cultivation of *E. coli* DH5 and *E. coli* BL21(DE3). Kanamycin was added to a final concentration of 50 mg/L during strain construction and fermentation.

1.4 Construction of Recombinant Plasmids

Using the synthesized colicin E7 gene (Colicin E7, GenBank: OYN48152.1) as template, the colicin E7 gene was amplified with primers E7-F and E7-R. The amplified E7 fragment and empty pET28a(+) plasmid were double-digested with Nco I and Xho I, ligated overnight using TaKaRa DNA Ligation Kit, transformed into DH5 competent cells, and the positive transformant was selected to obtain plasmid pET28a(+)-E7. Using laboratory-stored plasmid pKD46 containing the arabinose promoter as template, the arabinose promoter was amplified with primers PBAD-F and PBAD-R. The amplified arabinose promoter and pET28a(+)-E7 plasmid were double-digested with Sph I and Xba I, ligated overnight, transformed into DH5 competent cells, and the positive transformant was selected to obtain plasmid pET28a(+)-Ara-E7. Plasmids pET28a(+)-E7 and pET28a(+)-Ara-E7 were double-digested with Sph I and Bgl II to obtain T7-E7 and Ara-E7 expression cassettes. Empty pET28a(+) vector was double-digested with Sph I and Bgl II, and the vector fragment was ligated with T7-E7 and Ara-E7 cassettes. After transformation into DH5 competent cells and sequencing verification, the recombinant vectors were named pET28a(+)-T7E7 and pET28a(+)-AraE7. Using a laboratory-stored plasmid containing the red fluorescent protein (RFP) gene as template, the RFP gene was amplified with primers rfp-F and rfp-R. The amplified rfp fragment and plasmids pET28a(+)-T7E7 and pET28a(+)-AraE7 were double-digested with EcoR I and Sal I, ligated, transformed into DH5 competent cells, and after sequencing verification, the recombinant vectors were named pET28a(+)-T7E7-T7rfp and pET28a(+)-AraE7-T7rfp. Using a laboratory-stored plasmid containing the zearalenone degrading enzyme (ZENd) gene as template, the ZENd gene was amplified with primers ZENd-F and ZENd-R. The amplified ZENd fragment and pET28a(+)-AraE7 plasmid were double-digested with EcoR I and Sal I, ligated, transformed into DH5 competent cells, and after sequencing verification, the recombinant vector was named pET28a(+)-AraE7-T7ZENd.

1.5 Induction of Expression in *E. coli* Strains

Plasmids from correctly sequenced transformants were extracted and transformed into expression host *E. coli* BL21(DE3). Single colonies were inoculated into 5 mL LB liquid medium (containing 50 mg/L kanamycin) and cultured overnight at 37°C with shaking at 220 rpm. The next day, cultures were transferred to 30 mL LB liquid medium (containing 50 mg/L kanamycin) with 1% inoculation and grown at 37°C, 220 rpm to OD = 0.6-0.8. IPTG was added to a final concentration of 0.8 mmol/L, and induction was performed at 30°C, 180 rpm. For transformants containing the arabinose promoter, a second induction step was performed after 2 h of IPTG induction by adding L-arabinose to a final concentration of 2 g/L to induce lysis protein expression. During fermentation, samples were taken every 1-2 h to measure cell density (absorbance at 600 nm, OD).

1.6 SDS-PAGE Analysis

Samples taken during culture were centrifuged at 12,000 rpm for 2 min. For SDS-PAGE sample preparation, 40 L of culture supernatant was mixed with appropriate loading buffer. Alternatively, 400 L of culture supernatant was mixed with 400 L absolute ethanol, gently vortexed, and precipitated overnight at 4°C. The precipitate was collected by centrifugation at 13,000 rpm for 10 min, the supernatant was removed, and the white pellet was resuspended in 40 L PBS (pH 7.4) to achieve 10-fold concentration of the supernatant, then mixed with loading buffer for SDS-PAGE analysis.

1.7 Fluorescence Detection

Samples taken at various time points during culture were centrifuged at 10,000 rpm for 2 min. Then 200 L of culture supernatant was transferred to a black microplate, and red fluorescence intensity was measured using a fluorescence microplate reader with excitation at 530 nm and emission at 590 nm.

1.8 Zearalenone Degrading Enzyme Activity Assay

Fifty microliters of culture supernatant from *E. coli* BL21(pET28a(+)-AraE7-T7ZENd) was added to a reaction system containing 10 g zearalenone (ZEN) toxin, brought to 500 L with PBS (pH 7.4), and incubated at 37°C for 30 min or 60 min. The reaction was terminated by adding 50 L of 5 mol/L HCl, dried by evaporation, resuspended in 1 mL methanol, and analyzed by high-performance liquid chromatography (HPLC) with 10 L injection. Chromatographic separation was performed on a Waters XBridge® C18 column (5 m, 4.6 × 250 mm) with mobile phase of 50% acetonitrile and 50% water at a flow rate of 1 mL/min and column temperature of 30°C.

Results

2.1 Construction of *E. coli* Cell Lysis Systems

Colicin E7 acts on the inner membrane to alter membrane permeability and activates outer membrane phospholipase A, causing cell membrane damage [**?**]. Therefore, E7 expression in *E. coli* can achieve cell lysis. To construct the *E. coli* cell lysis system, we engineered two protein expression cassettes for recombinant protein and colicin E7 on the *E. coli* expression vector pET28a(+) (Figure 1 Figure 1: see original paper), enabling co-expression of both proteins within *E. coli* cells (Figure 1(b)). The target recombinant protein accumulates intracellularly, while E7 expression causes cell membrane damage and lysis (Figure 1(c)), thereby releasing the target recombinant protein into the culture medium (Figure 1(d)).

To construct an inducible lytic *E. coli* system, we inserted a colicin E7 expression cassette upstream of the T7 promoter in the pET28a(+) vector. The

plasmid construction process is shown in Figure 2 [Figure 2: see original paper]. Following the method described in Section 1.4, the synthetic E7 gene was inserted into the multiple cloning site of pET28a(+) to construct the T7-E7 cassette. The T7 promoter in the T7-E7 cassette was then replaced with the arabinose promoter to construct the Ara-E7 cassette. These two cassettes were individually inserted between the Sph I and Bgl II sites of empty pET28a(+) vector to create pET28a(+)-T7E7 and pET28a(+)-AraE7 vectors. To verify the function of the constructed *E. coli* lysis system, the red fluorescent protein gene *rfp* was inserted between the EcoR I and Sal I sites of both pET28a(+)-T7E7 and pET28a(+)-AraE7 vectors, generating pET28a(+)-T7E7-T7rfp and pET28a(+)-AraE7-T7rfp expression vectors.

2.2.1 Growth Curves of Inducible Lytic *E. coli* Strains

Recombinant *E. coli* strains harboring expression vectors pET28a(+)-T7E7, pET28a(+)-T7E7-T7rfp, pET28a(+)-AraE7, and pET28a(+)-AraE7-T7rfp were induced as described in Section 1.5. As shown in Figure 3 [Figure 3: see original paper], the OD of *E. coli* BL21(pET28a(+)-T7E7) and *E. coli* BL21(pET28a(+)-T7E7-T7rfp) increased briefly after IPTG addition then began to decline, indicating simultaneous induction of RFP expression and cell lysis. In contrast, the OD of *E. coli* BL21(pET28a(+)-AraE7) and *E. coli* BL21(pET28a(+)-AraE7-T7rfp) continued to increase rapidly at 30°C after IPTG addition, but began to decrease following arabinose addition, with culture clarification indicating cell lysis induced by arabinose.

2.2.2 Expression of Red Fluorescent Protein

Extracellular proteins (culture supernatants) from sampled cultures were prepared as SDS-PAGE samples according to Section 1.6. As shown in Figure 4 [Figure 4: see original paper], a protein band of approximately 30 kDa was observed in the extracellular fraction of *E. coli* BL21(pET28a(+)-T7E7-T7rfp) after IPTG induction (lanes 3–4, I-2 h and I-4 h), consistent with the expected size. This band was absent in uninduced *E. coli* BL21(pET28a(+)-T7E7-T7rfp) (lane 2, I-0 h) and in IPTG-induced *E. coli* BL21(pET28a(+)-T7E7) (lane 5, T7E7 I-4 h). Similarly, a ~30 kDa band appeared in the extracellular fraction of *E. coli* BL21(pET28a(+)-AraE7-T7rfp) after IPTG and arabinose induction (lanes 7–9, I-2 h, A-2 h, A-4 h), but was absent in uninduced cultures (lane 6) and in *E. coli* BL21(pET28a(+)-AraE7) induced with IPTG and arabinose (lane 10, AraE7 A-4 h). These results confirm successful construction of the *E. coli* cell lysis system, with RFP expressed and released extracellularly through cell lysis. Notably, the one-step induction system where T7 promoter controls both proteins showed lower RFP expression (Figure 4), whereas the two-step induction system with separate promoters for each protein yielded higher RFP expression, demonstrating that the IPTG-arabinose two-step lysis system is superior for efficient recombinant protein expression and extracellular release. This advantage arises because in the one-step system, target protein expression and cell lysis

occur simultaneously, preventing effective biomass accumulation, whereas the two-step system separates these processes, allowing substantial biomass accumulation before lysis.

2.2.3 Quantification of Red Fluorescence in Culture Supernatants

Red fluorescence intensity in culture supernatants was measured as described in Section 1.7. As shown in Figure 5 [Figure 5: see original paper], fluorescence was weak in supernatants before induction for both *E. coli* BL21(pET28a(+)-T7E7-T7rfp) and *E. coli* BL21(pET28a(+)-AraE7-T7rfp). After induction of cell lysis, the fluorescence intensity in supernatants from *E. coli* BL21(pET28a(+)-AraE7-T7rfp) was substantially higher—approximately five-fold greater—than that from *E. coli* BL21(pET28a(+)-T7E7-T7rfp), reflecting differences in RFP content and consistent with SDS-PAGE results.

2.2.4 Microscopic Observation of *E. coli* Morphology

Samples were taken during culture, resuspended, and examined under microscopy. As shown in Figure 6 [Figure 6: see original paper], both *E. coli* BL21(pET28a(+)-T7E7-T7rfp) and *E. coli* BL21(pET28a(+)-AraE7-T7rfp) exhibited intact rod-shaped morphology before induction (Figure 6(a), (d)). After IPTG and arabinose induction (Figure 6(b), (c), (e), (f)), rod-shaped cells were barely visible and numerous cell fragments appeared, confirming cell lysis.

2.3 Expression and Release of Zearalenone Degrading Enzyme in the *E. coli* Cell Lysis System

Results with RFP as a model protein confirmed successful construction of the *E. coli* cell lysis system, particularly the IPTG-arabinose two-step system, which enabled efficient expression and release of target protein into the culture supernatant. To further explore the system' s applicability, we attempted to express the zearalenone degrading enzyme (ZENd) gene.

2.3.1 Expression of Zearalenone Degrading Enzyme The ZENd gene was cloned from our laboratory stock and inserted between the EcoR I and Sal I sites of pET28a(+)-AraE7 to construct pET28a(+)-AraE7-T7ZENd. Expression was induced as described in Section 1.5, with samples taken every 1-2 h. Concentrated extracellular proteins (10-fold) from culture supernatants were prepared as SDS-PAGE samples. As shown in Figure 7 [Figure 7: see original paper], a protein band of the expected size was observed in the extracellular fraction of *E. coli* BL21(pET28a(+)-AraE7-T7ZENd) after IPTG and arabinose induction (lanes 3-7, I-2 h, A-1 h, A-2 h, A-4 h, A-6 h). This band was absent in uninduced *E. coli* BL21(pET28a(+)-AraE7-T7ZENd) (lane 2, I-0 h) and in *E. coli* BL21(pET28a(+)-AraE7) (lanes 8-9, AraE7 I-0 h and A-4 h). Some cell lysis and release of intracellular enzyme was observed in *E. coli* BL21(pET28a(+)-AraE7-T7ZENd) after 2 h of IPTG induction (before arabinose induction, lane

3), likely due to leaky expression of the araBAD promoter-controlled lysis protein in nutrient-rich LB medium. After arabinose induction for 1-2 h, most cells lysed and released large amounts of intracellular enzyme, resulting in abundant target protein in the supernatant (lanes 4–5), demonstrating that the *E. coli* cell lysis system can express ZENd and release it extracellularly.

2.3.2 Activity Assay of Zearalenone Degrading Enzyme Activity of ZENd released into the culture medium was assayed as described in Section 1.8. As shown in Figure 8 [Figure 8: see original paper], supernatants from before IPTG induction showed no ZEN degradation, while slight degradation was observed before arabinose induction, consistent with leaky expression of E7 from the araBAD promoter during LB culture, which induced partial cell lysis and release of small amounts of ZENd. This aligns with SDS-PAGE results (Figure 7, lane 3). As arabinose induction time increased, ZENd content in the supernatant rose, with ZEN degradation rates increasing from 6% to 58%. After 4 h of arabinose-induced lysis, ZENd was essentially fully released into the supernatant, which could degrade approximately 5.8 g of zearalenone toxin in 30 minutes at 37°C, and about 9.6 g in 60 minutes at 37°C.

Discussion

The *E. coli* expression system represents the earliest developed and most widely applied classical expression system in genetic engineering technology. Compared with other systems, *E. coli* offers numerous advantages for recombinant protein expression, including clear genetic background, high target gene expression levels, short cultivation cycles, and strong resistance to contamination. However, intracellular expression in *E. coli* entails high cell disruption costs, while extracellular secretory expression suffers from low secretion efficiency and limited applicability.

In this study, we constructed an *E. coli* cell lysis system by expressing colicin E7, enabling co-expressed foreign proteins to be released into the culture medium after intracellular expression and induced cell lysis, thus achieving rapid protein recovery. Using RFP as a model protein, we constructed two expression cassettes for E7 and RFP on the pET28a(+) vector. One system utilized the T7 promoter to simultaneously control expression of both E7 and RFP, requiring only a single induction to initiate expression of both proteins. The alternative system employed the araBAD promoter and T7 promoter to separately control E7 and RFP expression, enabling staged regulation of target protein expression and E7 expression. Comparative analysis of these two regulatory strategies on host growth, cell morphology, and recombinant protein expression revealed that staged induction of target protein and E7 expression by IPTG-arabinose yielded higher recombinant protein production and release into the medium. Since E7 expression is lethal to the host, the one-step induction system initiates target protein expression concurrently with E7-mediated cell lysis, preventing effective biomass accumulation and resulting in lower target protein yields. Building on

these results, we further explored the IPTG-arabinose two-step induction system for ZENd production, finding substantial expression and high activity in the culture supernatant, confirming the system's applicability for rapid intracellular protein release.

The two-step inducible cell lysis system constructed in this study offers dual advantages. First, because signal peptide adaptation for foreign proteins is unnecessary, the cell lysis system for extracellular protein release is essentially equivalent to the widely used *E. coli* intracellular expression system in terms of foreign protein expression, inheriting the broad applicability of *E. coli* intracellular systems for heterologous target proteins. Second, foreign proteins expressed intracellularly in *E. coli* can be released extracellularly through induced cell lysis, reducing disruption costs and simplifying protein purification, making it suitable for rapid screening of soluble protein mutant libraries.

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