The Case Study of Protein S Production

Abstract:

Customer supply: Gene sequence of protein S;
Protein properties: pI=5.30, about 23 KDa in size;
Service requirements: *E.coli* system, final concentration of the protein at 10 mg/ml, and purity of greater than 95%;
The provided experimental scheme by BIC: The protein expression level was verified via small-batch experiment, and then the goal was achieved through scale-up culture experiment and multi-step purification to meet the needs of customers.

1. Protein Expression and Identification

1.1 Plasmid construction & transformation

After gene synthesis, the expression vector was constructed and transformed into BL21 (DE3) competent cells. Subsequently, the sample was evenly coated on the LB plate containing corresponding resistance, which was then placed in the incubator at 37 ℃ overnight.

1.2 Preservation and induced expression of strain

The single cell clone was inoculated from the transformation plate, and added in three 4 mL LB media containing corresponding resistance. When OD_{600} was 0.5-0.8 during culture, IPTG with the final concentration at 0.5 mM was added to culture medium of test tube to induce expression.

1.3 SDS-PAGE identification of induced expression results

The bacterial cell was collected for sample preparation after induction of expression. The results of SDS-PAGE analysis were as follows:

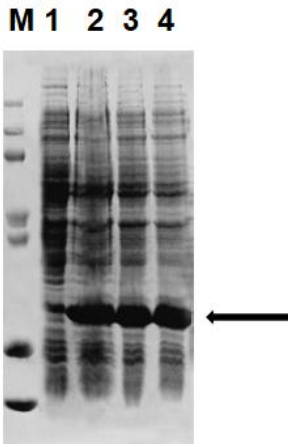

Fig. 1 SDS-PAGE analysis of protein expression

Lane M: Protein marker; Lane 1: Contrast; Lane 2: Induction at 15 ℃ overnight; Lane 3: Induction at 28 ℃ overnight; Lane 4: Induction at 37 ℃ for 4 h

1.4 Scale-up culture of protein S

According to the results of expression and identification of gel electrophoresis, 2-5 L expression bacteria containing protein S was involved for scale-up culture. After that, bacteria were collected, the cells were broken by ultrasonication and then centrifuged to obtain the supernatant (crushing and centrifugation at low temperature).

2. Protein Purification (The whole purification process was operated at low temperature)

2.1 The lysate supernatant was purified and the results were shown below:

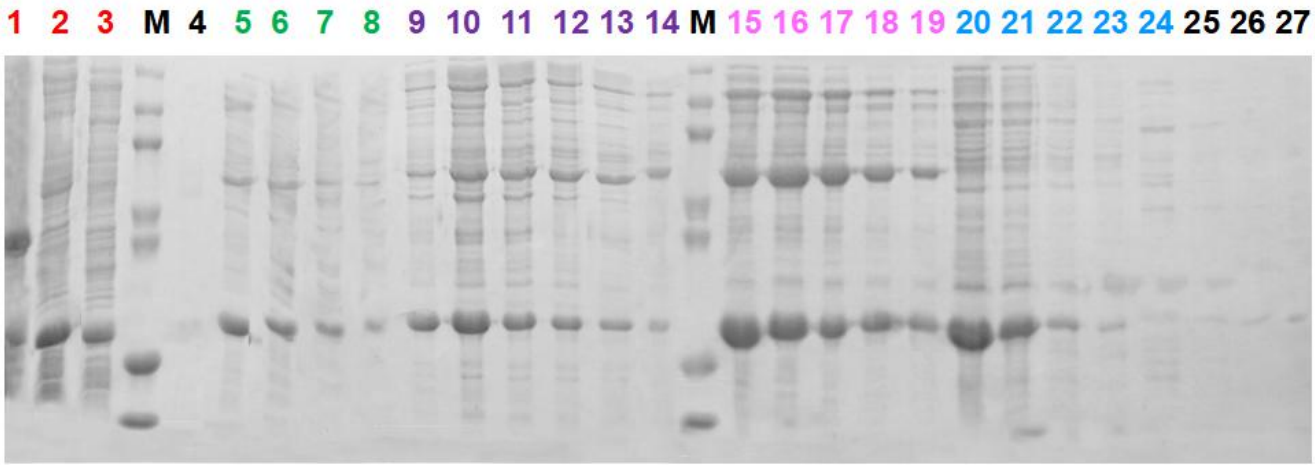

Fig. 2 SDS-PAGE before and after purification of the lysate supernatant

Lane M: Protein marker; **Lane 1**: Precipitation after centrifugation of total bacteria lysate; **Lane 2**: Collection of supernatant after centrifugation of total bacteria lysate; **Lane 3**: The effluent after incubation of Q column and supernatant; Lane4: The eluent of 20 mM NaCl; **Lane 5-8**: The eluent of 50 mM NaCl; **Lane 9-14**: The eluent of 100 mM NaCl; **Lane 15-19**: The eluent of 200 mM NaCl; **Lane 20-24**: The eluent of 300 mM NaCl; **Lane 25-27**: The eluent of 500 mM NaCl

Results: In addition to the target protein, the supernatant contained a certain amount of heterozygous protein.
Design solution: Flow-through was incubated with Q column again, and the initial salt concentration was increased from 20 mM to 50 mM.

2.2 The lysate supernatant of Flow-through (50 mM NaCl for equilibrium) was purified for the second time, as shown in the following figure:

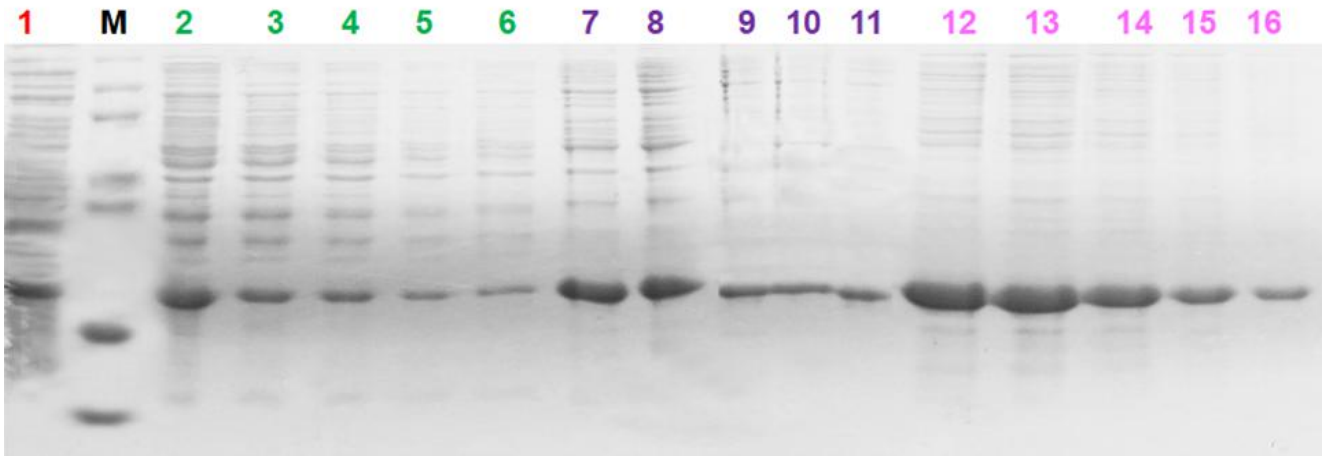

Fig. 3 SDS-PAGE of the lysate supernatant of Flow-through

Lane M: Protein marker; **Lane 1**: The effluent after incubation of Q column and supernatant; **Lane 2-6**: The eluent of 100 mM NaCl; **Lane 7-11**: The eluent of 200 mM NaCl; **Lane 12-16**: The eluent of 300 mM NaCl

Results: The elution effect of supernatant Flow-through (50 mM NaCl for equilibrium) has been improved, but it was still more complicated.
Design solution: Flow-through was continued to be incubated with Q column, and the initial salt concentration was increased to 100 mM.

2.3 The lysate supernatant of Flow-through (100 mM NaCl for equilibrium) was purified for the third time. The results were illustrated below:

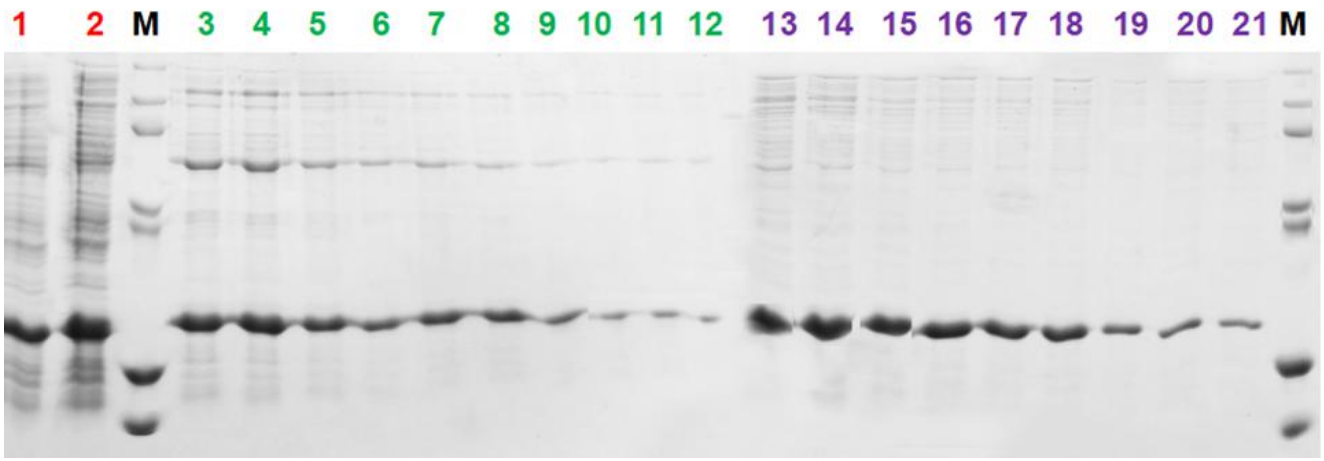

Fig. 4 SDS-PAGE of the lysate supernatant of Flow-through

Lane M: Protein marker; **Lane 1**: The supernatant of Flow-through (100 mM NaCl for equilibrium); **Lane 2**: The effluent after incubation of Q column and supernatant; **Lane 3-12**: The eluent of 200 mM NaCl; **Lane 13-21**: The eluent of 300 mM NaCl

Results: The elution effect of supernatant of Flow-through (100 mM NaCl for equilibrium) has been improved, which, however, was still not in line with the expectation.
Design solution: Further attempts to incubate Flow-through with DEAE column.

2.4 The lysate supernatant of Flow-through (100 mM NaCl for equilibrium) was purified for the fourth time. The results were described as follows:

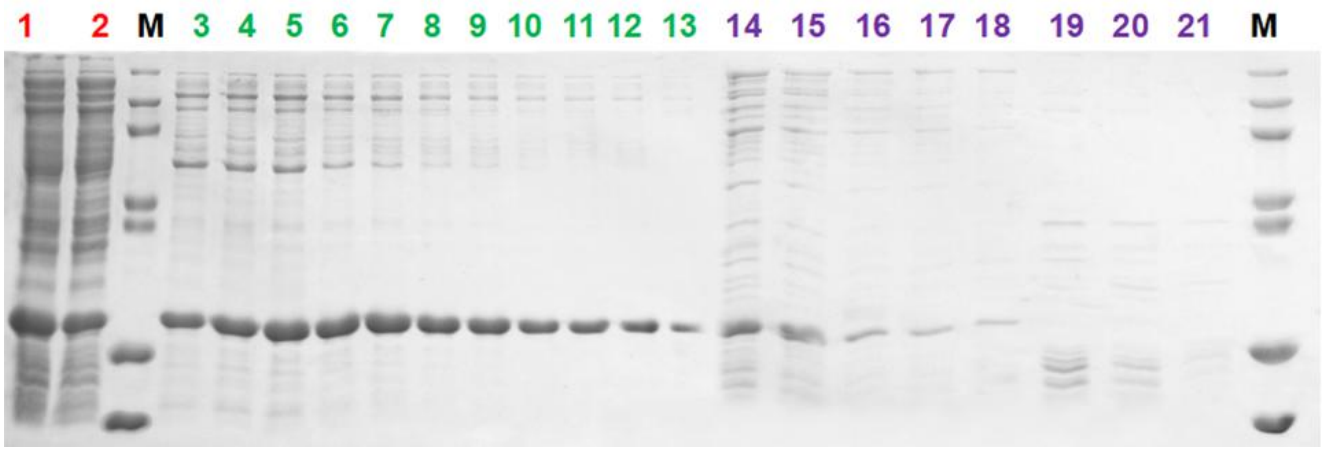

Fig. 5 SDS-PAGE of the lysate supernatant of Flow-through

Lane M: Protein marker; **Lane 1**: The supernatant of Flow-through (100 mM NaCl for equilibrium); **Lane 2**: The effluent after incubation of DEAE column and supernatant; **Lane 3-13**: The eluent of 200 mM NaCl; **Lane 14-21**: The eluent of 300 mM NaCl

Results: The elution effect of the supernatant of Flow-through (100 mM NaCl for equilibrium) was not significantly improved.
Design solution: The purified Flow-through of previous ones was collected and balanced by 200 mM NaCl, which was then incubated with DEAE.

2.5 The lysate supernatant of Flow-through (100 mM NaCl for equilibrium) was purified for the fifth time, with corresponding results shown as follows:

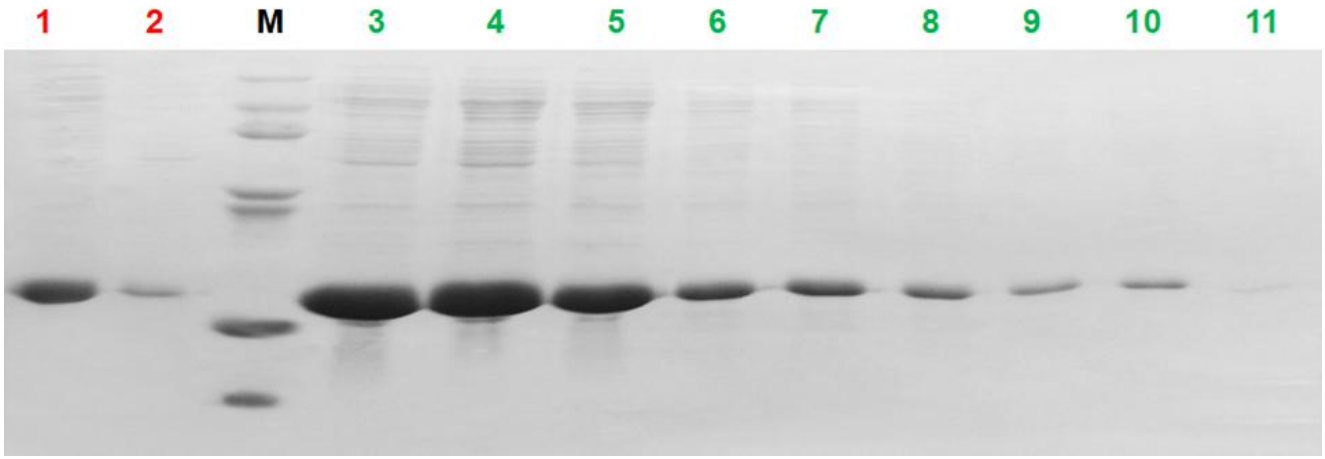

Fig. 6 SDS-PAGE of the lysate supernatant of Flow-through

Lane M: Protein marker; **Lane 1**: The supernatant of Flow-through (100 mM NaCl for equilibrium); **Lane 2**: The effluent after incubation of DEAE column and supernatant; **Lane 3-11**: The eluent of 300 mM NaCl

Results: The elution effect was still not ideal after collecting the supernatant.

Design solution: The next step was to collect the Flow-through in 3-9 and to eluent with molecular sieve after concentration.

2.6 The sixth purification was performed following the concentration of the collected Flow-through, and the results were presented below:

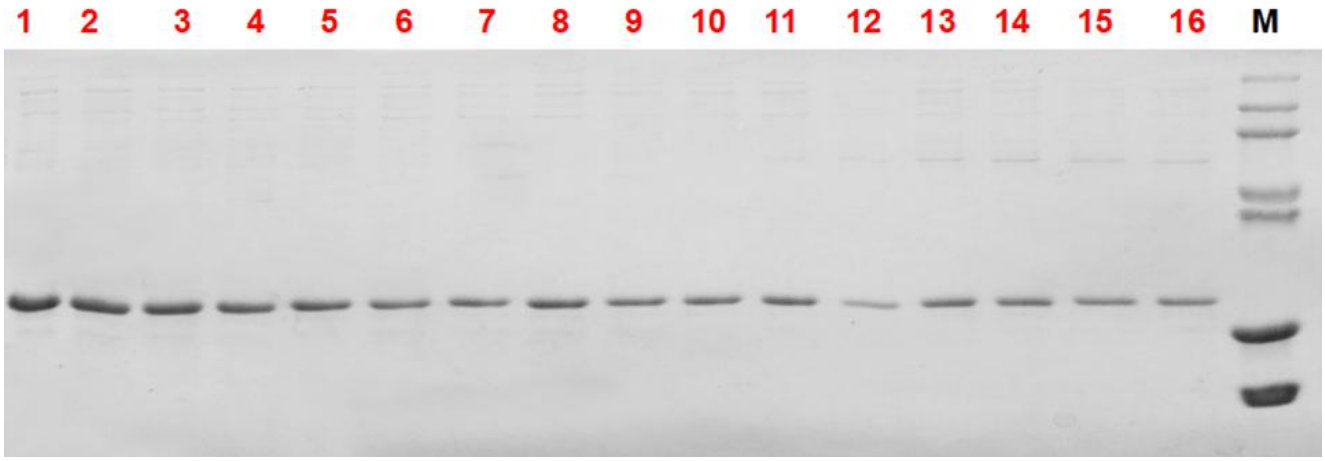

Fig. 7 SDS-PAGE for the purification of the collected Flow-through after concentration

Lane M: Protein marker; **Lane 1-16**: Elution and collection via molecular sieve

Results: The elution effect was satisfied after the concentration of the collected Flow-through, containing only a few heterozygous bands.

Next step: The Flow-through in 1-12 was collected, followed by package after concentration.

3. Protein Identification

Analysis of the freeze-thawing of protein S with BSA as the control, the results were shown below:

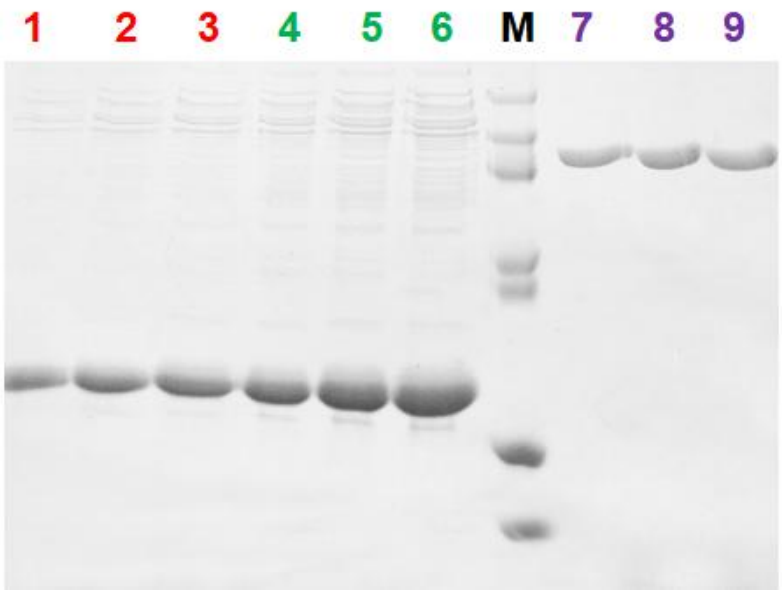

Fig. 8 SDS-PAGE electrophoretic results for analyzing the freeze-thawing of protein S with BSA as the control

Lane M: Protein marker; **Lane 1**: Protein S at the 20 times diluted concentration (1.2 ug); **Lane 2**: Protein S at the 20 times diluted concentration (1.5 ug); **Lane 3**: Protein S at the 20 times diluted concentration (2.0 ug); **Lane 4**: Protein S at the 10 times diluted concentration (2.2 ug); **Lane 5**: Protein S at the 10 times diluted concentration (4.4 ug); **Lane 3**: Protein S at the 10 times diluted concentration (6.4 ug); **Lane 7**: BSA (1.0 ug); **Lane 7**: BSA (1.5 ug); **Lane 7**: BSA (2.0 ug)

Results: The freeze-thawing of protein S was normal, with the concentration as high as 12 mg/ml and the purity of over 95%, which met the requirement of the customer. The protein would be arranged for delivery.