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Cloning and expression of African swine fever virus P32 and P72 recombinant proteins for diagnostic development

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Abstract

African Swine Fever (ASF) remains a global threat lacking commercial vaccines, necessitating precise diagnostic tools. This study focused on the cloning, expression, and purification of two key immunogenic ASFV structural proteins: P32 (CP204L) and the N-terminal P72 (B646L). Both proteins were expressed in *Escherichia coli* BL21(DE3) via 1.0 mM IPTG induction. Ni-NTA chromatography revealed that P32 was purified under native conditions, while P72 required denaturing conditions (8 M urea) followed by arginine-based renaturation. SDS-PAGE confirmed the successful purification of recombinant P32 (~30 kDa) and P72 (~45 kDa). These purified proteins will be utilized as diagnostic reagents in future ELISA development.

Keywords: African swine fever virus, P32, P72, Protein purification, Recombinant protein

Introduction

African Swine Fever (ASF) is a highly contagious, lethal hemorrhagic disease in pigs, often causing 100% mortality. It is caused by African Swine Fever Virus (ASFV), a complex dsDNA virus. In the absence of vaccines, effective control relies on diagnostics targeting key immunogenic proteins: P32 (CP204L), an early protein essential for viral internalization [1], and P72 (B646L), the major capsid protein used for genotyping and serology [2].

Materials and Methods

- **Gene Selection, Synthesis, and Cloning**

p32
PCR amplified

p72
Gene synthesis

Plasmid insertion
pET28a(+), pET32a(+)

Transformation
E. coli BL21 (DE3)

- **Optimization of Recombinant Protein Expression Conditions**

IPTG Induction
0.1, 0.2, 0.5, 1.0 mM
20°C, 25°C, 37°C

Auto-Induction
Autoinduction medium
20°C, 25°C, 37°C

- **Purification of Recombinant Proteins**

- **Native conditions**

P32 cell lysate → Purification of His-tagged Proteins via Ni-NTA

- **Denaturing conditions**

P72 cell lysate → Protein denaturing 8M Urea → Purification of His-tagged Proteins via Ni-NTA → Protein refolding by dialysis (Refolding Buffer + Arginine)

Results and Discussion

- **Construction of Recombinant Plasmids and Transformation**

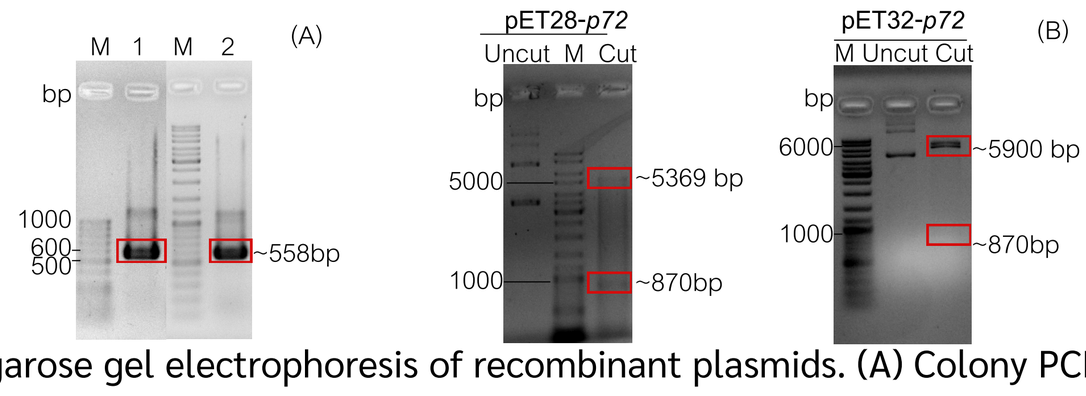

Fig. 1 Agarose gel electrophoresis of recombinant plasmids. (A) Colony PCR of *p32*. M: DNA marker; lanes 1–2: PCR products from pET28a-*p32* and pET32a-*p32*. (B) Restriction digestion of pET28-*p72* and pET32-*p72* showing expected fragments (~870 bp).

- **Recombinant Protein Expression**

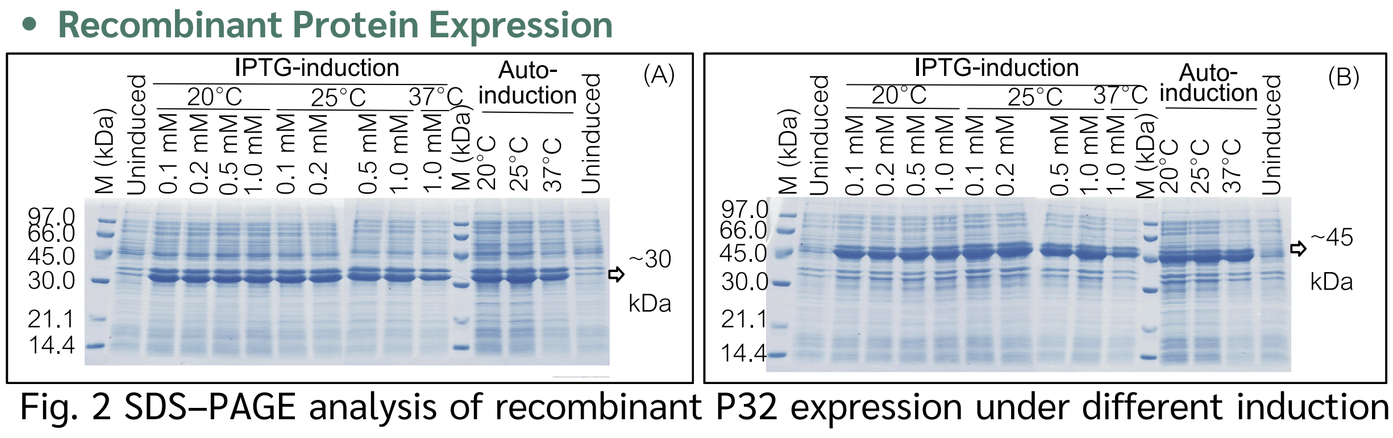

Fig. 2 SDS–PAGE analysis of recombinant P32 expression under different induction conditions. (A) *E. coli* harboring pET28a-*p32*; (B) *E. coli* harboring pET32a-*p32*.

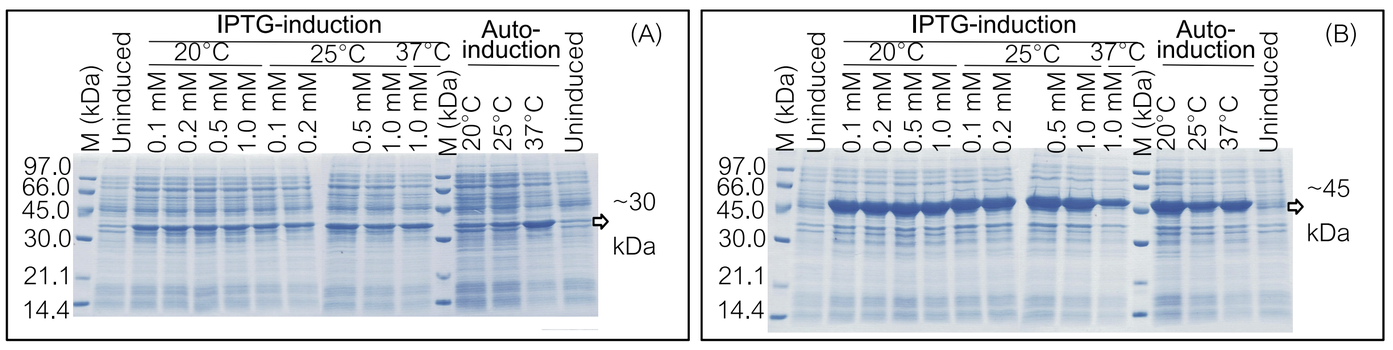

Fig. 3 SDS–PAGE analysis of recombinant P72 expression under different induction conditions. (A) *E. coli* harboring pET28a-*p72*. (B) *E. coli* harboring pET32a-*p72*.

- **Purification of Recombinant Proteins**

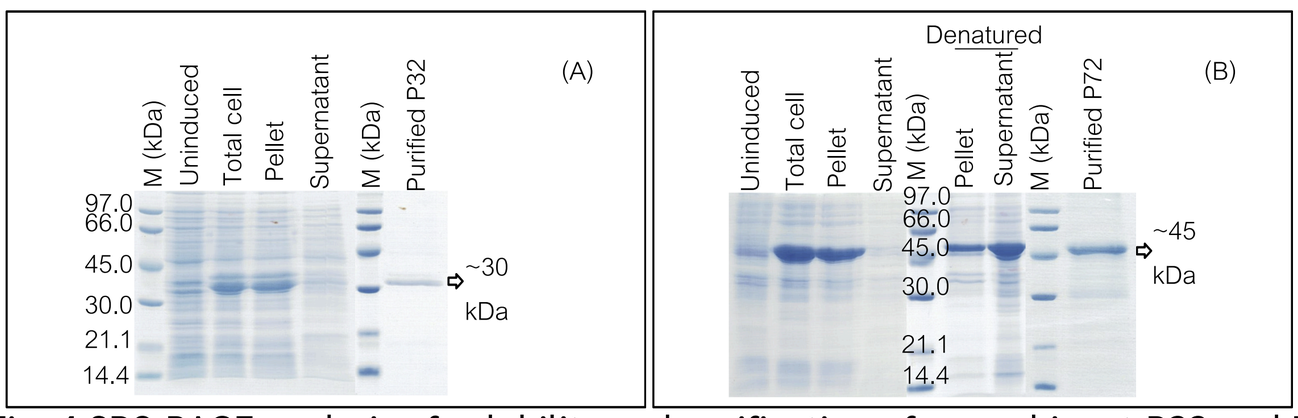

Fig. 4 SDS-PAGE analysis of solubility and purification of recombinant P32 and P72 expressed at 37°C for 4 h with 1 mM IPTG. (A) P32 (~30 kDa). (B) P72 (~45 kDa).

The high hydrophobicity of the P72 major capsid protein resulted in significant inclusion body formation, a common challenge for ASFV structural proteins involved in virion assembly [3]. Conversely, the improved protein solubility was attributed to the use of pET-32a and reduced induction temperatures. These strategies highlight the important role of thioredoxin tags as chaperone-like molecules in enhancing protein solubility in prokaryotic expression systems [4].

Conclusion

This study successfully expressed and purified ASFV P32 and P72 in *E. coli*. While P32 was recovered under native conditions, P72 formed inclusion bodies requiring denaturing and refolding strategies. These results highlight both the challenges and feasibility of expressing complex viral structural proteins in prokaryotic systems. The resulting purified recombinant antigens serve as essential reagents for enhancing serological diagnostics. Future studies will focus on the validation of these proteins in ELISA.

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