Expression and Identification of Poliovirus (Type 1) Immune Antigen in Escherichia Coli

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Abstract. This study aimed to achieve high-level expression of the key immunogenic epitope of poliovirus type 1 (PV1) VP1 in an *Escherichia coli* system and to characterize its antigenicity, thereby laying the groundwork for a safe, low-cost, and non-infectious subunit vaccine. A recombinant plasmid, pET-32a (+)-VP1, encoding the functional domain of PV1 VP1 was constructed and transformed into *E. coli* BL21(DE3). Following IPTG induction, the 43 kDa target protein was purified by Ni-NTA affinity chromatofigurey to >90 % homogeneity and shown to retain immunoreactivity. The established prokaryotic expression platform enables scalable production of PV-specific antigen, providing a robust technical resource for functional studies, immunogenicity evaluation, and next-generation vaccine development that will contribute to global polio eradication efforts.

Keywords: Poliovirus; VP1 Protein; Escherichia Coli; Prokaryotic Expression; SDS-PAGE.

1. Introduction

Poliomyelitis (commonly known as polio) is an acute infectious disease caused by the poliovirus, a member of the enterovirus genus within the Picornaviridae family. The virus primarily targets neurons, causing degeneration and necrosis that may lead to paralysis or even fatal respiratory muscle paralysis in severe cases. Humans are the viruss only natural host, with clinical manifestations including asymmetrical flaccid paralysis (hence the name "polio") after infection. Polioviruses are classified into three serotypes: 1, 2, and 3, with type 1 being the most common cause of paralysis. Most infected individuals experience asymptomatic infections, while some may develop symptoms like fever, headache, sore throat, and fatigue [1].

Polio is endemic throughout temperate regions, with higher incidence rates in summer and autumn, while tropical areas show similar seasonal patterns. The global polio prevalence has significantly decreased following the widespread use of oral polio vaccine (OPV). Currently, Chinas vaccination regimen primarily includes inactivated polio vaccine (IPV), bivalent oral poliovirus vaccine (bOPV), and combined vaccines containing IPV. However, OPV administration carries risks such as vaccine-associated paralytic poliomyelitis (VAPP) and vaccine-derived poliovirus (VDPV). Consequently, developing safer new vaccines has become a key focus of current research [2-3].

The varicella-zoster virus (VZV) particles exhibit an icosahedral symmetry structure, measuring approximately 20-30 nm in diameter and lacking an envelope. The viral capsid consists of four structural proteins: VP1, VP2, VP3, and VP4. Notably, VP1 serves as the primary surface protein, containing antigenic epitopes that induce neutralizing antibodies. This protein interacts with host cell receptors and plays a crucial role in viral pathogenesis [4].

In this study, the recombinant expression vector containing VP1 antigen epitope was constructed by molecular cloning technology [5], and the target protein was expressed and purified efficiently by using the E. coli expression system, which provided experimental basis for the subsequent subunit vaccine development.

2. Materials and Methods:

2.1 Materials:

2.1.1 Strains and Vectors

Escherichia coli DH5α (cloning strain), BL21(DE3) (expression strain); expression vector pET-32a (+)

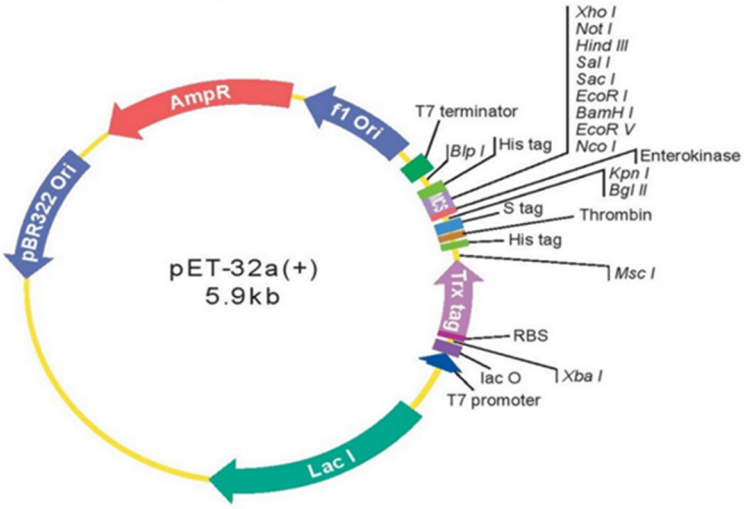
2.1.2 Reagents and Kits

Enzymes: T4 DNA ligase, restriction endonucleases (EcoRV, XhoI), Taq DNA polymerase; Kits: Standard agarose gel DNA recovery kit (TIANGEN DP209-02), non-toxic plasmid extraction medium (TIANGEN DP118-02); Media & Reagents: LB culture medium, kanamycin, IPTG, SDS-PAGE reagents, Coomassie Brilliant Blue stain, Ni-NTA affinity chromatofigurey column; Others: Broth culture medium, peptone, yeast extract powder.

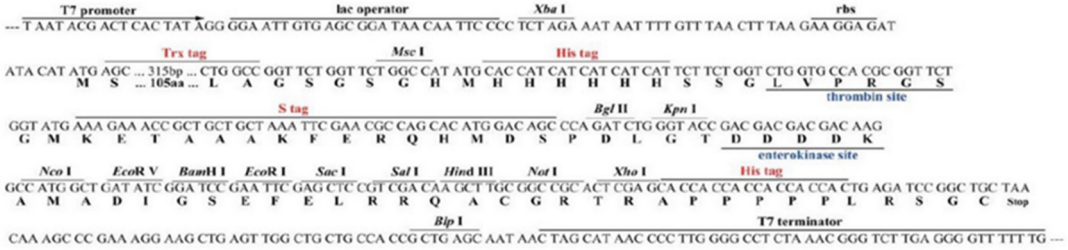
2.2 Experimental Method

2.2.1 Construction of Recombinant Expression Vector (Figure 1)

1) Target Gene Amplification: Design primers based on the VP1 protein epitope sequence of poliovirus, incorporating EcoRV and XhoI restriction enzyme cleavage sites at both ends, followed by PCR amplification of the target gene. 2) Restriction Enzyme Digestion and Ligation: Perform double digestion of the target gene and pET-32a (+)-vector using EcoRV and XhoI enzymes. Construct the recombinant plasmid pET-32a (+)-VP1 through T4 DNA ligase ligation. 3) Transformation and Screening: Transform the plasmid into DH5α competent cells. Screen positive clones using Kan-resistant agar plates. Verify the plasmid construct accuracy through restriction enzyme digestion and gene sequencing.



A. Expression vector map



B. Expression vector with multiple cloning sites

Figure 1. Recombinant expression vector

2.2.2 Plasmid Extraction and Agarose Electrophoresis

Refer to the instructions for the non-endotoxin plasmid extraction kit (TIANGEN DP118-02).

2.2.3 Reconstituted Protein Induced Expression

1) Preparation of Competent Cells: Prepare BL21(DE3) competent cells using the $CaCl_2$ method; 2) Transformation and Cultivation: Transform recombinant plasmids into BL21(DE3), pick single colonies, and inoculate them onto Kan-containing LB medium for overnight incubation at 37℃°C under shaking; 3) Optimization of Induction Conditions: Subculture to fresh LB+Kan medium at a 1:100 dilution ratio, test different IPTG concentrations (0.1-1 mM), temperatures (16℃,25℃,37℃°C), and induction durations (4-16 h), and screen for optimal expression conditions through SDS-PAGE analysis.

2.2.4 Protein Purification

1) Bacterial Cell Disruption: Collect the bacterial suspension cultured under optimal conditions by centrifugation at 4℃g. Resuspend in protease inhibitor-containing lysis buffer and perform ice-cryoprecipitation using sonication (power 300W,3s on/5s off, total duration 15-20 minutes). 2) Centrifugation and Separation: Centrifuge at 4℃g, 12,000 rpm for 30 minutes to collect the supernatant (containing soluble proteins). 3) Affinity Chromatofigurey: Load the storage buffer through gravity flow using a Ni-NTA preloaded column. Equilibrate the column with twice its volume of Binding/Wash Buffer at a flow rate of 0.5 mL/min to allow gradual buffer elution. Prepare the sample solution by mixing protein extract with Binding/Wash Buffer in a 1:1 ratio, ensuring the total volume is twice the column volume. Load the sample solution onto the column and collect the eluate into centrifuge tubes. Wash the column with twice its volume of Binding/Wash Buffer and collect the eluate. Elute histidine-tagged proteins from the column using twice its volume of Elution Buffer, repeating this step twice. Store each eluate separately until absorbance at 280 nm approaches baseline levels (as shown in Figure 2).

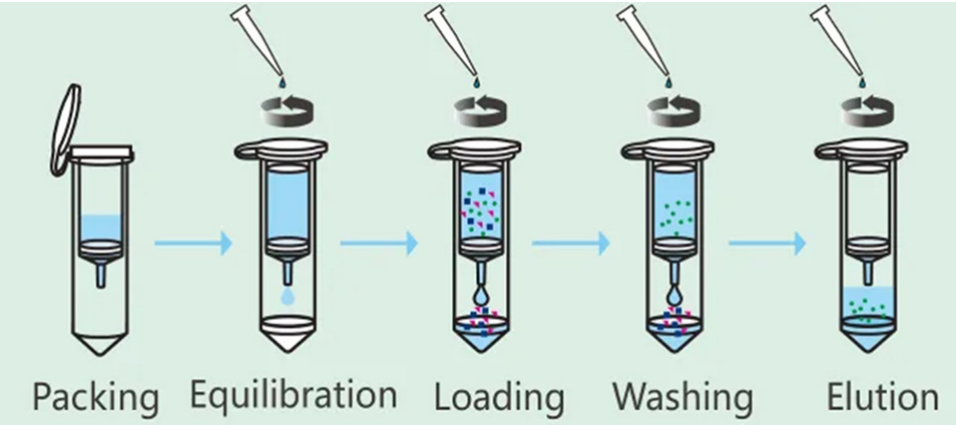


Figure 2. Affinity purification process of recombinant protein VP1

2.2.5 SDS-PAGE: Detection of Molecular Weight and Purity of Purified Protein;

3. Results and Analysis

3.1 Construction of Recombinant Plasmids

The restriction enzyme digestion results showed approximately 2 kb of vector fragments and target gene fragments (with expected sizes) after double digestion with EcoRV and XhoI enzymes, confirming the successful construction of the recombinant plasmid (Figure 2). Genetic sequencing revealed that the insert sequences of the recombinant plasmid pET-32a (+)-VP1 matched the expected sequences without any mutations or frameshifts (Figure 3).

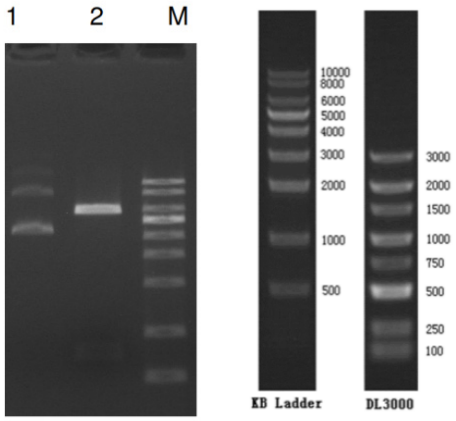


Figure 3. Electrophoresis results of double enzyme digestion verification of bacterial plasmid extraction
Lane M: KB Ladder
Lane 1: plasmid
Lane 2: plasmid digested by EcoRV and XhoI

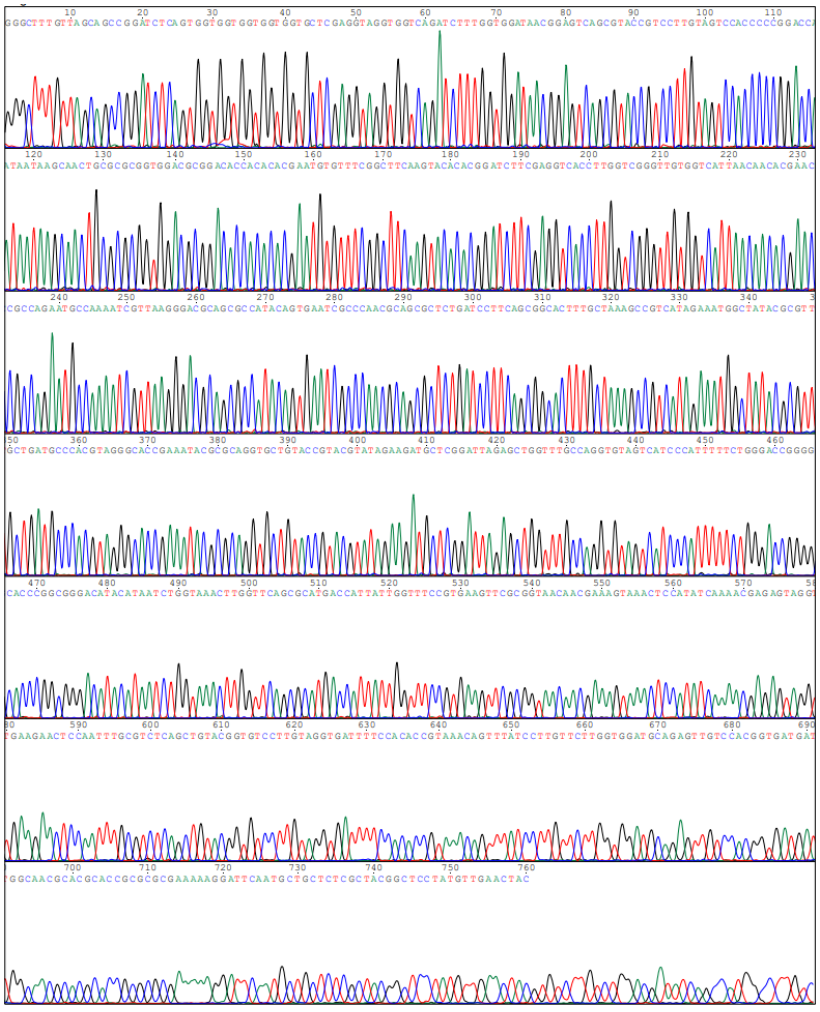


Figure 4. Chromosome sequencing report

3.2 Expression and Purification of Recombinant Protein

SDS-PAGE analysis revealed a specific band at approximately 40 kDa following IPTG induction, matching the theoretical molecular weight of VP1 fusion protein. The optimized expression

conditions were determined as: final IPTG concentration 0.5 mM and 16℃ induction for 16 hours, achieving optimal protein yield with high solubility. After Ni-NTA affinity chromatofigurey purification, the SDS-PAGE showed a single band with over 90% purity, and the protein concentration was measured at 1.2 mg/mL using the Coomassie Brilliant Blue method.

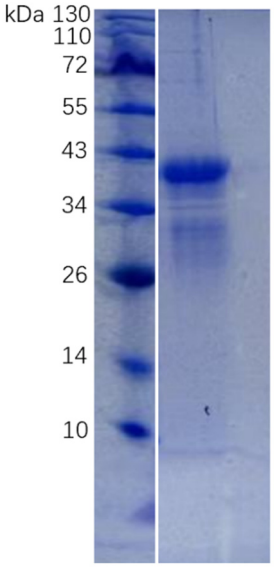


Figure 5. SDS-PAGE protein analysis

After ultrasonication and purification of IPTG-induced BL21(DE3) bacterial cells containing the recombinant plasmid, the SDS-PAGE results showed a specific band at molecular weight 43 kDa (Figure 5), matching the theoretical molecular weight. Following Ni-NTA purification, the protein purity exceeded 90% (Figure 5).

4. Conclusion

Through the construction of pET-32a (+)-VIP1 recombinant plasmid and optimization of expression conditions, this study achieved the high efficiency of PV1 VP1 protein expression in Escherichia coli (43 kDa) with purity>90%. This system laid a technical and material foundation for the development of polio subunit vaccine.

From a technical perspective, the T7 promoter system of pET-32a (+) vector can efficiently induce protein expression, and the N-terminal and C-terminal labeling sequences simplify the process of protein purification and identification. The optimization of induction conditions (such as prolonging the induction time at low temperature) effectively improves the solubility of protein and reduces the formation of inclusions.

Compared with existing studies, this research focuses on key epitopes of VP1 protein rather than complete viral particles, which reduces the risk of nonspecific reactions and avoids the VDPV risks associated with oral poliovirus vaccine (OPV) and production limitations of inactivated poliovirus vaccine (IPV). However, it should be noted that proteins expressed in Escherichia coli lack glycosylation modifications found in eukaryotic cells, potentially affecting their immunogenicity. Subsequent animal experiments are required to verify their ability to induce neutralizing antibodies.

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