Original Article

Cloning and Expression of Recombinant Nucleoprotein of Influenza H1N1

Somaie Tavakoli[1], Mojgan Bandehpour[2,3*], Zeinab Soleimanifar[3], Mehdi Goudarzi[4], Bahram Kazemi[2,3]

[1] Department of Biochemistry, Faculty of Basic Sciences, Payame-noor University, Tehran, Iran
[2] Cellular & Molecular Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Department of Biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background: Influenza virus is the major cause of lower respiratory tract illnesses on the worldwide. Vaccination can be an effective tool to prevent its outbreak. Highly conserved viral nucleoprotein is an effective vaccine candidate to provide heterosubtypic immunity, offering resistance against various influenza virus strains.

Materials and Methods: In present research *NP* gene was inserted in pET-22b expression vector. New construct (pET-22b/NP) was transformed into *E. coli* BL21 (DE3) strain and the expression of nucleoprotein was induced by IPTG. It was analyzed by SDS-PAGE and confirmed by Western blotting.

Results: Western blotting confirmed the expression and production of recombinant Influenza nucleoprotein.

Conclusion: These results suggest that the codon-optimized influenza A virus *NP* gene can be efficiently expressed in *E. coli*.

Keywords: Influenza virus, Cloning, Expression, Nucleoprotein gene

***Corresponding Author:** Mojgan Bandehpour, Biotechnology Department, School of Medicine, Cellular & Molecular Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Email: m.Bandehpour@sbmu.ac.ir; Bandehpour@gmail.com

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Introduction

It is well known that influenza infections cause severe illness in 3–5 million people worldwide and kill 250,000–500,000 humans each year. Despite the currently influenza vaccines are against subtype-specific virus and produce neutralizing antibodies, there are still significant deficiencies, because the designed vaccines cannot protect against new subtypes or antigenic variants[1].

Influenza A nucleoprotein (NP), the protein component of ribonucleoprotein (RNP) complexes, is also relatively conserved that make it an appropriate candidate for an efficient influenza vaccine. Its real function is the protection of single strand RNA genome of influenza virus against degradation by various enzymes[2,3]. Influenza virus NP characteristically has a nuclear localization signal (NLS) and an RNA-binding domain. Influenza virus nucleoprotein (NP) is more conserve than its membrane glycoproteins[4,5]. Although the NP induces an antibody response. Horimoto et al. (2005), in a study conducted on influenza pandemics, found out that the nucleoprotein induces a $CD8^+$ T-cell response which may contribute to resistance against severe disease following influenza A virus infection[2]. According to Ruan (2012) recombinant influenza nucleoprotein can induce specific antibodies and T cell

responses in mice[6]. So our purpose in present research was to produce the sufficient recombinant NP in the *E. coli* host.

Methods

The preparation of the *NP* gene construct

1500 bases nucleotide sequences of the influenza H1N1 NP (A/Shiraz/14/2010) was obtained from the NCBI. According to the available data, the NP gene was considered for codon optimization using the Encorbio website:
(www.encorbio.com/protocols/codon.htm), and then it was artificially synthesized (MWG,Germany) and inserted into a pGE plasmid.

To sub- cloning of *NP* gene into expression vector, it was digested with *NotI* and *HindIII* (Fermentas, Lithuania) restriction enzymes. The digestion product was analyzed by electrophoresis on 1.5% agarose gel and the desired band was removed and purified by DNA gel extraction kit (Qiagene, USA). The purified fragment was inserted into digested pET22b vector (Pasteur, Iran) and transformed into *E. coli Top 10* strain. Recombinant plasmid was extracted by plasmid extraction kit (Qiagene, USA). The recombinant plasmid was confirmed by PCR analysis using universal primers and also digestion by *NotI* and *HindIII* restriction enzymes[7].

The expression of the NP protein

Extracted recombinant plasmids were transformed into *E. coli BL21* by Hanahan method. Overnight incubated colonies were inoculated in LB broth. Cultures in the logarithmic phase (at OD600 of 0.6) were induced for 6 hour with 1 mM, IPTG (Isopropyl β-D-1-thiogalactopyranoside). After induction cells were lysed in 5x sample buffer [100 mM Tris HCl pH 8, 20% (w/v) glycerol, 4% (w/v) SDS, 2% (w/v) beta-mercaptoethanol, 0.2% (v/v) bromo phenol blue] (sigma) and analyzed on 12% (v/v) SDS-PAGE. The resulting gel was stained with coomassie brilliant blue R-250. The uninduced control culture was analyzed in parallel[7].

The western blot analysis

Result of SDS-PAGE was confirmed by western blotting using Anti-His Tag polyclonal antibody as the primary antibody and anti-mouse HRP conjugated immunoglobulin (abcam, UK) as the secondary one[8].

Results

NP gene construct confirmation

The 1500 bases NP gene was extracted from pGE vector and ligated into pET-22b expression vector. The recombinant plasmid and NP gene (1500 bases) after digestion have been shown in figure 1. Figure 2 shows analysis of new construct by PCR reaction.

NP recombinant protein expression

The expression of recombinant influenza nucleoprotein in *E. coli BL21 (DE3)* was detected and compared with control bacteria by SDS PAGE analysis which was shown in figure 3.

The confirmation of protein expression by western blot analysis

The result of expressed band was confirmed by western blotting. The brown band for positive expression was appearing 5 hours after induction (Figure 4).

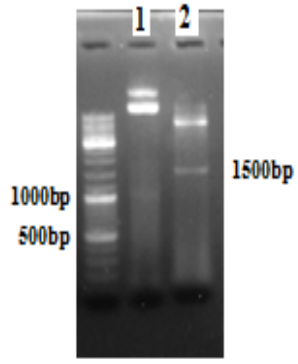

Figure 1. Restriction analysis of pET22b/NP by *NotI* and *HindIII*; Lane 1: undigested recombinant plasmid, Lane 2: digested recombinant plasmid with *NotI* and *HindIII*.

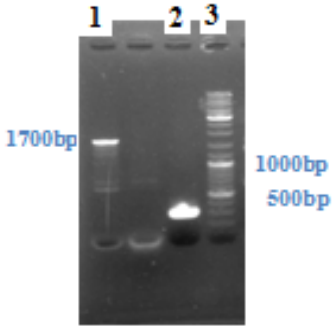

Figure 2. Confirmation of pET22b/NP by PCR; Lane 1:1700bp PCR product of cloned plasmid, Lane 2: 200bp PCR product of intact plasmid, Lane 3:100 bp DNA ladder.

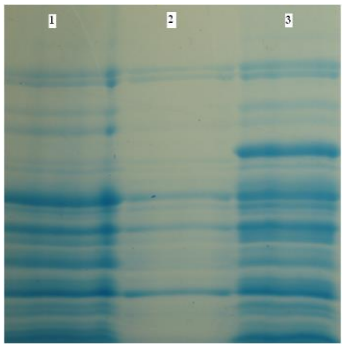

Figure 3. Comparison between protein expressions in different times after induction; Lane 1: lysate of bacterial cell content pET-22b, Lane 2: lysate of Host cell (control), Lane 3: lysate of expressed recombinant bacteria 5 hours after induction by IPTG.

Figure 4. Conformation of the expressed NP by Western blot analysis.

Discussion

The purpose of the study was to develop of Influenza H1N1 recombinant nucleoprotein production. Influenza viruses cause highly contagious respiratory diseases with potentially fatal outcomes[9]. Pandemic influenza represented a major threat to global public health. The results of this study showed that pET-22b expression vector and induction with IPTG were the appropriate condition for influenza NP production. The NP gene is highly conserved compared with the other genes of virus[10]. Influenza A nucleoprotein (NP), the major protein component of ribonucleoprotein (RNP) complexes, is also relatively conserved making it an attractive candidate for a universal flu vaccine[1]. By attention to mentioned issues in this study, the codon optimized recombinant nucleoprotein (rNP) of H1N1 influenza virus of Iran isolate strain was sufficiently expressed using a pET expression system. Most amino acids are encoded by more than one codon and each organism carries the 61 available amino acid codons. Studies of the genomes of a wide variety of organisms have revealed a correlation between gene expression level and codon usage bias, namely that high gene expression leads to high bias[11]. So the set up condition is proper to express the influenza NP gene in BL21 (DE3) plysS *E. coli* host.

Also Salahuddin and Khan's study[12] showed that mutations in essential functional regions of conserved influenza NP and NS1proteins lead to reduced expression of these genes in vitro. Therefore, distinct internal secondary structures of viral mRNA may be important for viral gene expression. Finally, introducing mutations in viral genes while preserving their secondary RNA structure, suggests a new method for the generation of appropriate expressed recombinant proteins[13].

The protective vaccines against all influenza A viruses might substantially reduce severity of infection and limit spread of disease during outbreaks[9]. It is recommended that the design of a multi conserved domains protein against of Influenza is a necessary thing in the world.

Conclusion

In the present study we tried to express NP recombinant protein of influenza A H1N1 virus (A/Shiraz/14/2010 strain) in order to provide preventive strategies for the Iranian population. In the present research a 50 KD band was observed which is in agreement with previous studies. Based on the results of this study, it could be concluded that the NP gene of the influenza A H1N1 virus (A/Shiraz/14/2010 strain), could be cloned and the rNP (recombinant NP) could be expressed using the bacterial protein translation system. Since this protein is a conservative protein among influenza A viruses, it can be used as a potent vaccine for the prevention of various types of pandemics caused by influenza A.

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