The Quarterly Scientific Journal of Applied Biology

Vol.37, No.1, Sering.79, Spring 2024, p. 6-9

Journal homepage: https://jab.alzahra.ac.ir

doi.org/10.22051/jab.2023.42860.1543

Isolation, Cloning and Expression of T7 RNA Polymerase from T7 Bacteriophage in *E. coli*

Leyla Davarvash, Maghsoud Pazhouhandeh* and Mohammad Ahmadabadi

Department of Biotechnology, Azarbaijan Shahid Madani University, Tabriz, Iran

***Corresponding author: pazhouhandeh@gmail.com**

Introduction

Bacteriophage T7 belongs to the *Podoviridae* family of viruses that attacks specifically gram-negative bacteria such as *E. coli*. Bacteriophage Basically, phages have facilities in their genome so that they can express their genes in the host bacteria and fulfill their needs and multiply. Among these, the T7 promoter with a sequence of 23 nucleotides has been identified in this phage, which can be expressed in *E. coli* and is widely used in genetic engineering in various expression systems.

For any RNA synthesis, it is enough to put the desired gene in a plasmid against the T7 promoter, and then this plasmid will produce a large amount of RNA of the desired gene in one hour at 37°C, in presence of buffer and rNTP and T7 RNA polymerase enzyme. RNAs synthesized by T7 RNA polymerase enzyme are used in studying the structure and function of RNA, in hybridization experiments such as Southern blot, microarray, RNAi, antisense experiments, and inoculation and infection of RNA viruses. By cloning of the gene of this enzyme in a suitable plasmid and transferring it into the bacterial host cell, a high amount of this enzyme can be produced (Nilsen et al., 2013). Due to the importance of T7 RNA Pol enzyme in biotechnology, in this research, isolation of T7 RNAPol gene from bacteriophage T7 and construction of appropriate expression vector for its expression were carried out. This research is actually the background for creating a commercial production line of T7 RNA Pol enzyme.

Material and Methods

In this research, the T7 RNA Polymerase gene was isolated from T7 bacteriophage DNA and cloned into the pGEX2TK expression plasmid (Pazhouhandeh et al, 2006) to prepare for its

commercial production. For this purpose, bacteriophage T7 DNA (Sigma 69390-M) was used to clone the T7 RNA polymerase gene (GenBank: M38308.1) with a length of 2877 nucleotides.

The primers used to perform PCR: (Eurogentec). Forward primer sequence (BamHI) was 5'-aaGGATCCatgaacacgattaacatcgcta-3' and reverse primer sequence (EcoRI) was 5'-aaGAATTCttacgcgaacgcgaagtccga-3'. The cutting sites embedded in these primers are underlined. After cloning, plasmid extraction was done by miniprep method and the plasmids were confirmed again by PCR and after RNase treatment, pGEX-T7RNAPol recombinant plasmids were sent for sequencing (University of Lille, France). *E. coli* bacteria of Top10 and Rosetta competent cells were used for cloning and expression of T7 RNA polymerase protein, respectively.

After confirming the sequence of pGEX-T7RNAPol recombinant plasmids, one of these plasmids was transferred to the Rosetta expression strain by electroporation, and after selection of colonies on ampicillin-containing medium, one colony was cultured for overnight at 37°C. The main liquid LB medium containing ampicillin was inoculated to a volume of 500 ml with 1 ml of the pre-culture and after the culture density reached OD_{600}=0.6-0.8, induction with IPTG to a final concentration of 1 mM was done. After 6 hours of induction, the bacterial culture was centrifuged for 15 minutes at 5000 rpm at 4°C. The culture medium was removed and the cells were suspended in extraction buffer. Ultrasonic sonication (Bandelin) was performed on the existing solution. By setting 40% power for the device, 10 pulses were introduced during 15-second time cycles to break the bacterial cells. After sonication, protein extraction was performed by removing the upper phase after centrifugation for 15 minutes at 14000 rpm at 4°C. At the same time and in parallel, cultivation, induction and sonication were also done on the bacterial sample containing an empty plasmid without genes. The resulting protein was precipitated with 100% acetone and kept for 2 hours at -70°C with a 14000 rpm centrifuge, and after washing with 80% acetone, it was finally dissolved in 30 µl of PAGE buffer. Before protein electrophoresis, the samples were heated for 5 minutes at 95°C and prepared to run on the gel. Protein electrophoresis was performed in SDS-PAGE and the gel was stained with coomassie blue solution and then decolorized with 10 % acetic acid.

Result and Discussion

First, using the registered sequence of T7 RNA polymerase gene, its specific primers were designed and it was amplified from phage DNA by PCR, after optimizing the temperature conditions. The purified PCR product along with pGEX2TK plasmid were digested with BamHI and EcoRI enzymes. Then, in order to clone the T7 RNA polymerase gene, the plasmid and gene were used for the ligation reaction. After performing the ligation reaction, transformation of *E. coli* by electroporation method was done. In the next step, to confirm the recombination of the plasmid in the colonies grown on the selective medium, the PCR reaction with the specific primers

of the T7 RNA polymerase gene with the same initial conditions was performed on 16 of these grown colonies. The PCR products, confirmed the 2877 bp band in some selected colonies. The recombinant plasmid was again confirmed by PCR on the extracted plasmids from bacteria. Also, the recombinant plasmids were digested by BamHI and EcoRI enzymes to be confirmed moreover. Based on the predictable map for the recombined plasmid, it was expected that two fragments with the sizes of 2877 and 4969 bp would be created by digestion and the electrophoresis profile showed them.

The recombinant plasmid, then, were sent for sequencing to the university of Lille (France) and the resulted sequences were aligned and compared using BLAST and Multialign online software with the T7 RNAPol gene registered sequences in NCBI (accession number M38308). The pGEX2TK-T7RNAPol plasmids number 3 and 7 showed the correct and complete matching of the sequence. One of them was transferred in parallel with the empty pGEX2TK plasmid to the competent cells of *E. coli* (Rosetta strain) by electroporation.

After selecting the positive colonies and reconfirming with PCR in order to extract the protein from the bacteria, liquid pre-culture and then the main culture was done, and after induction time with IPTG, the culture medium was removed by centrifuge and the cells exposed to sonication. The bacterial protein was extracted and precipitated with acetone. Finally, 30 µl of PAGE buffer was added to the sediment at the bottom of the tube. The result of SDS-PAGE electrophoresis after staining with coomassie blue showed that T7 RNA polymerase protein has been well expressed from this plasmid in this bacterial strain. Because this protein is produced in pGEX2TK plasmid as a fusion with GST, its molecular weight (97 kDa) is increased by about 24 kDa and is seen in the range of 120 kDa on the gel.

Conclusions

T7 RNA polymerase enzyme is one of the most important and widely used enzymes in biotechnology and molecular biology laboratories. This enzyme is used in RNA synthesis and in vitro transcription. The commercial kits of this enzyme are expensive and imported to Iran. Considering the increasing importance of this enzyme, its mass and low-cost production in Iran is of particular importance. In this research, efforts were made to isolate and to clone the T7 RNA polymerase gene (M38308), into an expression plasmid, pGEX2TK, in order to produce the enzyme. Cloning of T7 RNA polymerase gene was done by Digestion-ligation method. the resulted plasmid, pGEX-T7RNAPol, was confirmed by PCR, enzymatic digestion and sequencing and then transformed into Rosetta strain of *E. coli*. The T7 RNAPol protein expression in this bacteria was performed by inducing with IPTG and after extraction, it was confirmed by SDS-PAGE. This research is a prerequisite for T7 RNA Polymerase commercial and mass production.

Keywords: T7 RNA polymerase, T7 bacteriophage, Transcription, Expression system, Recombinant proteins.

Acknowledgement

The financial support of Azerbaijan Shahid Madani University is gratefully acknowledged.

Declaration of conflict of interest

We declare that this manuscript is original and has not been published before and is not currently being considered for publication elsewhere. No conflicts of interest associated with this publication. We confirm that the manuscript has been read and approved for submission here by all the named authors.

Statement on ethics

None materials and methods used in this research need ethics statement.