Original Article

Optimization of Taq DNA polymerase enzyme expression in *Escherichia coli*

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Abstract

Background: In the present study, we optimized the experimental conditions using pET-15b expression vector to obtain large amounts of Taq DNA polymerase.

Materials and Methods: Correct framing of the gene in the expression vector pET-15b and its orientation were analyzed by digestion and sequencing. Production of Taq DNA polymerase in *Escherichia coli* BL21 (DE3) cells was induced by incubation with different concentrations of IPTG. Optimum production occurred with the addition of 1 mM IPTG for 2 h. The activity of the obtained enzyme was measured by comparing the intensities of the produced DNA bands in PCR reactions.

Results: Recombinant plasmid containing taq polymerase gene was confirmed by restriction digestion and DNA sequencing. Purified protein was identified by Western blotting. Optimum condition for the production of the enzyme was induction with 1 mM IPTG for 2-3 h. Addition of NP-40 increased enzyme stability.

Conclusion: We expressed the recombinant Taq DNA polymerase in *E. coli* using a T7-based promoter system and obtained an active and stable enzyme.

Key words: Cloning, expression, optimization, pET-15b, Taq DNA polymerase

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INTRODUCTION

Taq DNA polymerase has been used for PCR and other related techniques for many years. This enzyme was first isolated from hot water springs bacteria but later due to high demand, many investigators studied its production in bacteria using recombinant DNA technology.[1-4]

Engelke *et al.* reported the production and purification of Taq polymerase in *Escherichia coli*.[5] In another study, Pluthero has suggested a rapid purification technique for the isolation of this enzyme.[6] Other investigators have also studied techniques to produce pure enzyme in high quantities. These differ in the type of vectors used, methods of purification, and conditions for the induction of enzyme production.[4-6]

In our laboratory, the gene for Taq polymerase enzyme

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was cloned and placed in the expression vectors pUC18 and pET15b.[7] These vectors have the advantage of producing large quantities of this enzyme in *E. coli* using IPTG as an inducer. However, our preliminary results demonstrated that pUC18 was not as efficient as pET15b for producing Taq DNA polymerase. Therefore, in the present study, we optimized the experimental conditions for the production of large amounts of active enzyme using the pET-15b expression system.

MATERIALS AND METHODS

Bacterial strains and plasmids

E. coli XL1-Blue strain was obtained from Cinnagen Company (Iran). pET 15b plasmid was purchased from Novagen Company (Germany). Taq-pTZ57R was constructed in our pervious study.[7]

Amplification of Taq DNA polymerase gene

Recombinant Taq-pTZ57R plasmid was purified using High Pure Plasmid Isolation Kit (Bio Rad, Germany) and was utilized as the template for PCR amplification of Taq DNA polymerase gene. The sequences of forward and reverse primers were: 5′-AGTCCATA TGCTGCCCCTCTTTGAGCC-3′and 5′-AGGGGA TCCATCACTCCTTGGCGGAG- 3′, respectively. PCR amplification was performed using the following reagents: 1 X PCR reaction buffer (50 mM KCl, 20 mM Tris- HCl (PH 8.4), primers (each 2.5 μM), 3 mM $MgCl_2$, 0.5 mM dNTPs, 0.4 μg template DNA, 1X Q solution, 5 Unit Taq polymeras (QIAGEN, Germany). The final reaction volume was 50 μl. PCR cycles were as follows:

One cycle of 5 min at 94°C, 35 cycles of: l min at 94°C, 2 min at 55°C, 3 min 72°C, and one cycle of 20 min at 72°C.

Cloning of Taq DNA polymerase gene into pET-15b expression vector: Restriction enzymes

The amplified Taq polymerase gene was digested with *Nde*I and *Bgl*II restriction enzymes. For the digestion of the vector, *Nde*I and *Bam*HI restriction enzymes were utilized. These were then electrophoresed and extracted from the agrose gel using QIA Quick Gel Extraction Kit (QIAGEN, Germany).

Ligation was performed under the following conditions: DNA insert (30 ng), 14 ng vector, 1units of ligase enzyme, 1X ligase buffer. The reaction mixture was incubated overnight at 16ºC. The ligated mixture was used for the transformation of *E. coli* XL1-Blue and BL21 (DE3) $CaCl_2$ treated competent cells using heat shock method (42°C, 90 s).[8] Plasmid extraction was performed using High Pure Plasmid Isolation Kit (Bio Rad, Germany). Restriction enzymes, *Hind III, Xba I and EcoR I* were used for the digestion of the prespared plasmids (1 h, 37ºC). Subsequently, purified plasmids containing Taq DNA polymerase gene was sequenced (Kowsar Bbiotech Company, Iran).

Expression of Taq polymerase gene

Overnight cultures of the recombinant *E. coli* BL21 (DE3) / rpET-15b plasmids were cultured overnight in LB medium containing ampicillin (100 μg/ml). When the bacteria reached their semi-growth phase (OD_{660}=0.4), induction of Taq DNA polymerase was performed using different concentrations of IPTG (0, 0.25, 0.5, 1, 1.5 Mm) and samples were shook for 0, 1, 2, and 3 h at 37°C.

Purification and electophorsis (SDS-PAGE) of Taq polymerase enzyme

Enzyme purification was accomplished by the Desai Method (4). A 12% polyacrylamide gel was used for electrophoresis and Coomassie blue R-250 (0.1 %) was used for gel staining.[9]

Western Blotting

After electrophoresis of proteins, they were transferred into nitrocellulose membrane (500 mM, 45 min). Since the cloned gene was placed into pET15b vector with a poly His sequence attached to its 5´ end, anti His antibody and ECL method was used for the detection of the produced Taq DNA polymerase enzyme.[9]

Assay for the measurement of Taq DNA polymerase enzyme activity

Determination of the enzyme activity was performed using PCR with reagents containing produced Taq DNA polymerase, genomic DNA of *Bacillus subtilis*, and primers for the amplification of the lipase gene.[10] PCR cycle was as follows: One cycle of 5 min at 94°C, 35 cycles including: 94°C for 1 min, 55°C for 2 min,

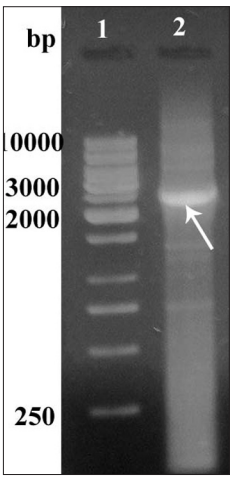

Figure 1: PCR amplification of Taq DNA polymerase gene. Lane 1: Gene Ruler DNA ladder. Lanes 2: 2800 bp PCR products (indicated by arrow).

72°C for 3 min and one cycle at 72°C for 20 min.

RESULTS

PCR amplification of Taq DNA polymerase gene produced a 2800 bp DNA fragment [Figure 1]. The pattern of the digested DNA bands were as follows: *Hind* III two bands (2180 and 4138 bp), *Xba* I one band (8200 bp), and *Eco*RI one band (8200 bp) [Figure 2]. Some of these plasmids were sequenced and the presence of the gene and its orientation was confirmed.

The production of Taq polymerase in *E. coli* BL21 cells was induced by IPTG. Using Nickel columns, this enzyme was purified and its isolation was confirmed by Western blotting using anti-His antibodies [Figure 3]. The effect of the time of incubation with IPTG was also investigated [Figure 4]. As shown in this figure, maximum production of Taq DNA polymerase was obtained after 2 h of incubation with 1 mM IPTG.

The activity of the obtained Taq DNA polymerase was analyzed by comparing the intensities of the PCR amplified DNA bands with those produced by the commercial enzyme [Figure 5].

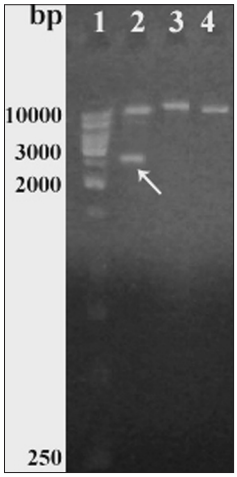

Figure 2: Restriction digestion of the amplified TaqDNA polymerase gene. Lane1: Gene Ruler DNA ladder. Lane 2: Hind III digestion, two bands at 2180 and 4138 bp. Arrows show 2180 bp band. Lane 3: XbaI digestion, band at 8200 bp. Lane 4: EcoRI digestion, band at 8200 bp.

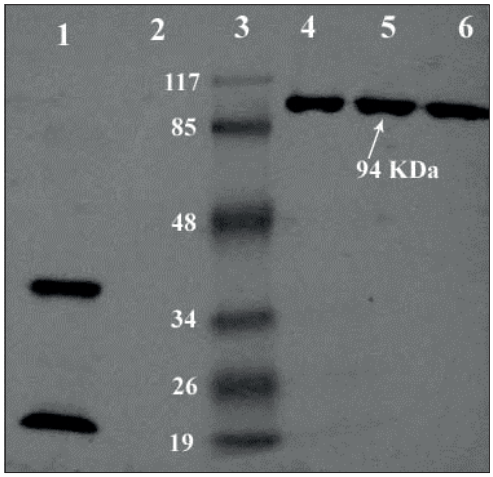

Figure 3: Identification of the recombinant Taq DNA polymerase enzyme by Western blotting using anti-His antibody. Lane 1: Control, expressed pET15b plasmid in *E. coli* BL21 cells. Lane 2: Control, *E. coli* BL21 (DE3) crude lysate. Lane 3: Protein molecular weight marker Lanes 4, 5, and 6: Expressed and purified Taq DNA polymerase in BL21 (DE3) bacteria. Arrow shows 94kDa Taq DNA polymerase band

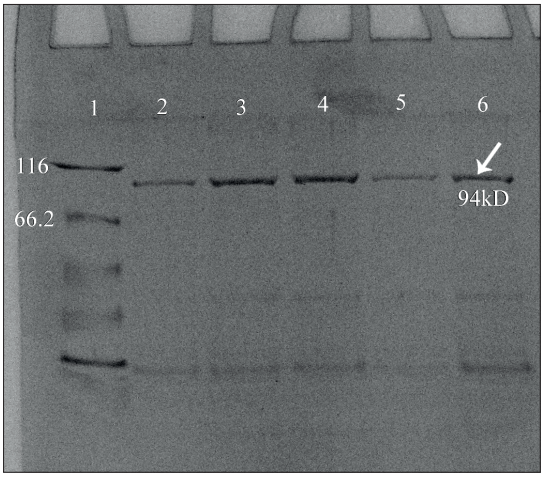

Figure 4: Optimization of the expression of the recombinant Taq DNA polymerase by altering the time of induction with IPTG. Lane 1: Protein molecular weight marker. Lanes 2, 3, 4, 5 and 6: SDS-PAGE of purified recombinant plasmids expressed in *E. coli* BL21 (DE3) cells after induction with 1 mM IPTG for 1, 2 and 3 hours. Arrow shows 94kDa Taq DNA polymerase band

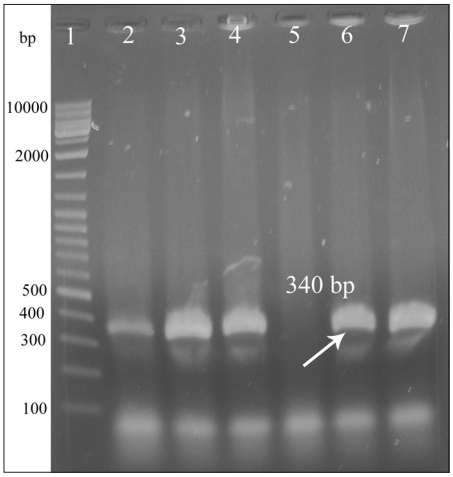

Figure 5: Evaluation of the activity of the recombinant Taq DNA polymerase. Lane 1: Gene Ruler DNA ladder. Lane 2, 3, 4, and 6: PCR amplification of the B. subtilis Lipase A gene using purified recombinant Taq polymerase. Arrow shows the presence of the 340 bp amplified lipase gene. Lane 7: PCR amplification of the B. subtilis Lipase A gene using a commercial Taq DNA polymerase.

DISCUSSION

Many investigators have studied the production and optimization of Taq DNA polymerase enzyme expression in bacteria. Some of these data are not available since they are either patented or not released by the manufacturers. Desai and Pfaffle used pUC18 plasmid for the cloning and expression of this enzyme.[4] The expression was induced by incubation with 0.5 mM IPTG for 16-20 h. The authors reported a high level of enzyme production although their system was leaky and expression occurred even before the addition of the inducer.[4] On the other hand, Engelke *et al.* utilized pTTQ18 vector for the cloning and expression of Taq DNA polymerase.[5] The use of TAC promoter had the advantage of exerting more control over the expression of this enzyme.[2,11] Other investigators also have selected systems similar to what was mentioned above.[12,13] On the other hand, in our study, the pET expression system was used. The advantage of this system is the use of T7 promoter and BL21 cells which can selectively produce T7 RNA polymerase enzyme. Our results indicated that the optimum expression of Taq DNA polymerase occurred after 2 h of incubation with IPTG as the inducer. This shorter incubation time allows us to produce higher amounts of the enzyme in a unit of time as compared to the above mentioned studies. Additionally, the enzyme produced in our study was stable (data not shown). This stability may be due to the method used for its purification.

Regarding the activity of the produced recombinant enzyme, the results were variable. In some experiments, the activity was similar to the commercial enzyme while in others the activity was low. Addition of NP-40 to the purification solution helped but did not eliminate this variability. Manzur *et al.* dialyzed the purified Taq DNA polymerase in two steps for 3 days. This procedure possibly eliminated the interfering materials and thus resulted in a consistent activity for the enzyme.[2] In our study, this procedure and other experiments need to be performed in order to further improve the quality of the produced Taq DNA polymerase enzyme.

In conclusion, we expressed the recombinant Taq DNA polymerase in *E. coli* using a T7-based promoter system and obtained an active and stable enzyme. This enzyme can be used in our laboratory for PCR reactions. However, it is now possible to study the structure-function relationship of this enzyme by using site-directed mutagenesis techniques.

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