

Production of recombinant expansin and detection by SDS page analysis in *Escherichia coli*

Serap Gunes¹  • Demet Ektiren¹  • Mehmet Karaaslan²  • Hasan Vardin² 

¹ Dicle University, Diyarbakir Vocational School of Agriculture, Department of Food Processing, Diyarbakir, Türkiye

² Harran University, Faculty of Engineering, Department of Food Engineering, Şanlıurfa, Türkiye

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Correspondence: Serap Gunes

E-mail: serap.gunes@dicle.edu.tr



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Abstract

The study aims to produce Expansin protein isolated from a young tomato plant by using *Escherichia coli* which is used in recombinant protein production. Continuous culture is the most common method used to grow cells for recombinant protein production. In the study, the K12 strain of *E. coli* was used as a culture for the production of Expansin protein. The used LeExp1 gene was isolated from a young tomato plant. Since the related gene is found in very small amounts in plants, it has been reproduced using the PCR method and has been made workable with this method. T17 vector (T7 RNA polymerase system), which is frequently used in the production of recombinant protein, was used as the bacterial expression vector. The T7 RNA polymerase system is a commonly used vector in *E. coli*. With the transfer, the *E. coli* bacterium was given the ability to produce recombinant protein. Whether the obtained recombinant protein expressed the appropriate protein was determined by SDS Page analysis.

Keywords: Recombinant protein, Expansin protein, LeExp1 gene, *Escherichia coli*, PCR

INTRODUCTION

Proteins are a group of macromolecules with various biochemical functions that are widely used for research and applications in fields such as the health and food industry, mainly thanks to biotechnological advances (Andersson et al., 2018; Rabert et al., 2013; Walsh et al., 2014). While obtaining protein from natural sources is very laborious and expensive, the efficiency of obtaining protein in these ways is low, but advances in DNA technology have made it possible to use expression systems more efficiently for recombinant protein production (Garcia-Fruitos, 2014; Gräslund et al., 2008; Sanchez-Garcia et al., 2016).

Today, the first publication on the creation of recombinant DNA and the replication of recombinant DNA in the cell, which comes to mind in the field of modern biotechnology, was published in 1972 and 1973 (Jackson et al., 1972; Katartaş, 2011). The first genetically modified DNA molecule was developed using recombinant DNA technology; It was obtained by Paul Berg in 1972 (Hughes, 2001). As a result of these developments, with the understanding of the importance of Recombinant DNA technology, companies using this technology (Genentech, Eli Lilly and Company) were established. The result of the studies was first carried out on the *E. coli* bacteria in 1978. It has been described that a new strain capable of producing insulin has been synthesized by performing a genetic manipulation on the bacterium (Ladisch et al., 1992).

Expansin proteins are a family of proteins in the plant cell wall structure. Expansin proteins are divided into four subgroups: Expansin A, Expansin B, Expansin A-like

and Expansin B-like. Expansin proteins were first isolated from the cucumber plant by Daniel Cosgrove in 1992. The two Expansin proteins revealed in the cucumber plant correspond to an average of 270 amino acids and their molecular weights are 29 kDa (kiloDalton) and 30 kDa. In general, A and B Expansins and Expansin-like proteins consist of approximately 300 amino acids and have molecular weights of around 25-28 kDa (Cosgrove, 2000). Expansin proteins have an important role in developmental processes in plant cell development, fruit softening, root hair growth, stigma and pollen tube development, and cell wall fragmentation (Hartley, 2006). Enzymes that break down the cell wall are used in many industries, such as the biofuel production, cellulose industry and the food industry.

Expansin proteins are found in very small amounts in plants and there is no microbial production of these proteins. In addition to the fact that the expansive proteins cannot be produced in pure culture, it is very costly to isolate these proteins from plants. Many of these problems can be eliminated by producing expansive proteins in the form of recombinant proteins. With the production of the recombinant Expansin protein, an alternative protein capable of modifying the cell wall structure can be obtained in addition to the enzymes that degrade the cell wall. To facilitate gene studies, the gene can be isolated and amplified. The method of isolating and amplifying a gene is to clone it by inserting the relevant gene into another DNA molecule that acts as a vector or tool that can be replicated in a living cell. It is the formation of a new DNA molecule

Table 1. Forward and Reverse Primers

Primer name	Length	Melting temperature (°C)	GC%	Sequence (5'-3')
TOF1	24	62.2	33.3	CACCATGGGTATCATAATTTTCAT
LeExpR1	20	63.3	50	GCAGCCACTTCAACCTTCT

as a result of combining these two DNAs of different origins. Although genetic events such as crossing over (change of parts in chromosomes) technically provide the formation of recombinant DNA, DNA molecules formed by the participation of segments obtained from different biological sources are generally used for this work. The recombinant DNA molecule is introduced into a prokaryotic or eukaryotic host cell. The host cell then makes copies of the existing DNA molecule and replicates the vector with its foreign DNA fragment. Foreign DNA is amplified with these developing processes and these amplified DNAs are purified for further analysis (Mullis, 1990).

In the study, the LeExp1 gene isolated from the tomato plant was amplified by PCR method and transferred to the T17 expression vector (T7 RNA polymerase system) by a suitable kit. Vector *E. coli* was transformed into bacteria in liquid culture and *E. coli* bacteria were given the ability to produce recombinant protein.

MATERIALS AND METHODS

Materials

Young tomato (*Solanum lycopersicum*) seedlings were used as the relevant gene source in the study. Tomato seedlings were grown in special capped plastic boxes in the medium without air. They were left to grow for 15 days. Gene DNA and RNA obtained from the grown seedlings were used as a source.

E. coli strain K12 and vector T17 were commercially available from Invitrogen. DNA restriction and DNA ligase enzymes required for the cloning process were obtained from New England Biolabs. The IPTG inducer and the necessary medium required for the research were obtained from Sigma company. The gene of interest in the study was selected based on the gene sequences stored in the NCBI (National Center of Biotechnology Information) database.

PCR Primer Design

Designing Forward and Reverse PCR primers used to amplify the gene of interest is a critical step for expression. Depending on the use of vectors, when designing PCR primers, the sequence should be known to facilitate cloning, and the N-terminal and C-terminal peptide tags should be known for cloning the PCR product (Anon., 2010).

Forward and Reverse Primers Used

The forward and reverse primers used in the study are shown in the Table 1.

DNA Extraction from Tomato Plant

DNA isolation method was performed as stated by Doyle and Doyle (1990). The method used in the research is an extraction process based on CTAB (Cetyl trimethylammonium bromide).

Blunt End PCR Product Production

The production of the blunt PCR product used in the study was carried out by taking into account the basic molecular biology rules (T. Maniatis, J. Sambrook, E.F. Fritsch). The extracted LeExp1 gene was amplified using a Biorad branded thermocycler. According to this method, the components were mixed in the PCR tube. PCR conditions for the prepared PCR mixture were determined as 3 minutes at 94 °C, 30 seconds at 94 °C, 60 °C, and 72 °C for 35 cycles, and finally 10 minutes at 72 °C. The duplication process was carried out taking into account the conditions. After this process, the PCR mixture was run on a 0.8% agarose gel and visualized.

Purification of LeExp1 Gene from PCR Reaction

After the PCR reaction, the DNA was purified. Purification was performed using the GE Healthcare GFX PCR DNA and Gel Band Purification Kit. The liquid phase obtained as a result of purification was stored at -20 °C.

Cloning of LeExp1 Gene into TOPO Vector and Transformation into Chemical Competent Cells

After obtaining the desired PCR product, it was cloned into the pET TOPO vector. The recombinant vector TOP10 obtained by TOPO cloning was transformed into *E. coli* One Shot. When performing directional TOPO cloning, the molar ratio between the PCR product and the TOPO vector is critical for the reaction to be successful. To obtain high efficiency in the TOPO cloning reaction, the PCR product: TOPO vector ratio should be between 0.5:1 and 2:1. The ratio of PCR product and TOPO vector used in the study is 1:1. The cloned LeExp1 gene was transferred to the pET100/D TOPO expression vector by the Champion kit used in the study. The recombinant vector TOP10 obtained as a result of the TOPO cloning reaction was transformed into *E. coli* One Shot cells.

Isolation of Plasmid DNA from *E. coli* Cell and Transformation of Plasmids into Expression Cells

Isolation of plasmid DNA from *E. coli* cells and transformation of plasmids into expression cells were performed following the analytical steps specified in Champion™ pET100 Directional TOPO® Expression Kit.

Recombinant Protein Expression

E. coli cells must reach a certain concentration to produce recombinant protein. Cells were incubated for 4 hours in a 37 °C shaker until the OD reached 0.5-0.8. When the bacterial density reached the desired level, the culture was divided into two 5 ml tubes. IPTG was added to one of the tubes containing the cultures at a final concentration of 0.5-1 mM (0.0005-0.001 M). The tube with the other culture was left for control purposes without adding anything. Incubation was continued in a 37 °C shaker for the expression process. Marking was done for each culture at a time interval of 4-6 hours. The samples were centrifuged in a centrifuge to remove the liquid phase and the pellets were stored at -20 °C.

RESULTS AND DISCUSSION

Extraction and purification of nucleic acids is the first step in most molecular biological studies and all recombinant DNA techniques. The purpose of nucleic acid extraction is to purify nucleic acids from different sources for specific analysis using PCR analysis.

Control of DNA Extraction by PCR

In the study, the nucleic acids used to isolate the LeExp1 gene encoding Recombinant Expansin proteins were isolated from young tomato plants. For this purpose, DNA coding for the LeExp1 gene was extracted from

the genome of the tomato plant. Analysis by gel electrophoresis was performed to check whether the isolated nucleic acids could be used in molecular studies. 0.8% agarose gel image of DNA of LeExp1 gene is given in Figure 1. PCR samples used in the study were prepared using appropriate primers. The region multiplied by the primers used in the study is 991 bp. However, according to the gel image results, the DNA size was approximately 1.3 kb. It is predicted that the reason for this may be the coexistence of meaningful and meaningless regions.

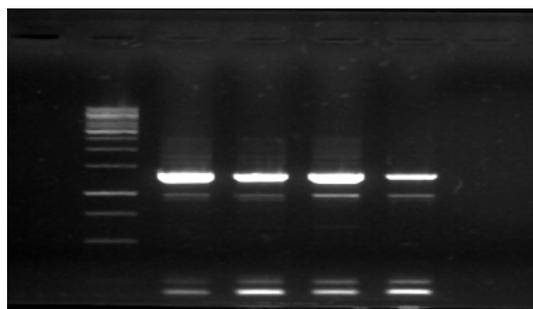


Figure 1. DNA marker (1st Well), 0.8% agarose gel image of DNA of LeExp1 (2nd - 5th well) gene

Control of Purification of the LeExp1 Gene

Purification was performed following the procedure using the GFX PCR DNA and Gel Band Purification Kit. To control the efficiency of the purification process, the obtained DNA was visualized by running on 0.8% agarose gel. The obtained electrophoresis gel image is given in Figure 2. The band size seen in Figure 2 is approximately 991 bp and it has been determined that the purification process has been done successfully according to the gel image.

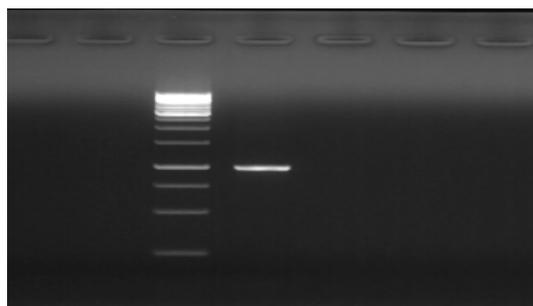


Figure 2. DNA marker (1st well), 0.8% agarose electrophoresis gel image of DNA belonging to LeExp1 (2nd – 5th well) gene to control the efficiency of the purification process

Control of Plasmid DNA Isolation

PCR analysis was performed to check whether the plasmid DNA isolation was performed effectively and whether the LeExp1 gene was inserted into the TOPO vector. The image of 0.8% electrophoresis gel obtained as a result of the analysis is given in Figure 3. In the electrophoresis gel image, there is a DNA marker in the first well, an A100 TOPO vector in the second well, and an

A200 TOPO vector in the third well. It is known that the size of the vectors is approximately 1000 bp according to the marker used and that special primers are used to amplify the DNA in the vector. Since the size of the DNA used is approximately 1000 bp, the result is successful in both wells, that is, the DNA has been successfully inserted into the vector. Since the band of the A100TOPO vector is displayed more sharply in the electrophoresis gel image, subsequent analyzes were continued with this vector.

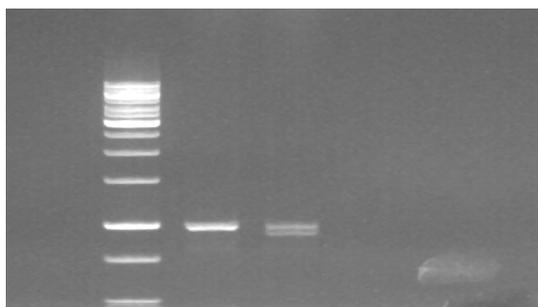


Figure 3. Plasmid DNA isolation 0.8% electrophoresis gel image, DNA marker (well 1), LeExp1 gene (well 2)

SDS Page (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) Analysis

Samples in tubes left for expression were collected every 5 hours, which continued for approximately two days until expression was complete. SDS Page analysis was performed to detect the presence of protein in the samples stored at -20 °C during the expression process. The gel image obtained as a result of the analysis is given in Figure 4. The gel image seen in Figure 4 is an image of the plasmid in protein synthesis without the addition of an inducer. The samples in the wells of the gel belong to the 0, 10, 20, 30, 40, and 45 hours, respectively. The size of the LeExp 1 protein is known to be approximately 30 kDa. The size of the bands observed as marked in corresponds to approximately, also 30 kDa.

Protein synthesis was continued by adding an IPTG inducer to observe whether the IPTG inducer positively affected protein synthesis. In the gel image shown in Figure 5, protein synthesized plasmids were visualized by adding an IPTG inducer. As a result of the use of IPTG at a concentration of 0.1 M in protein synthesis, it was observed that its synthesis improved positively.

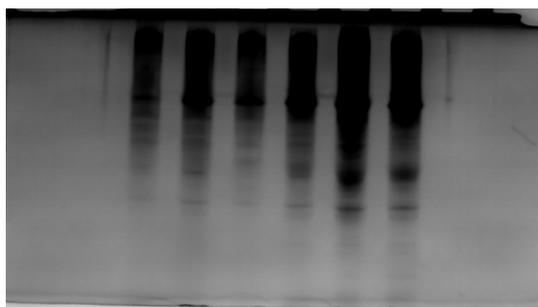


Figure 4. SDS Page gel image of Vector A

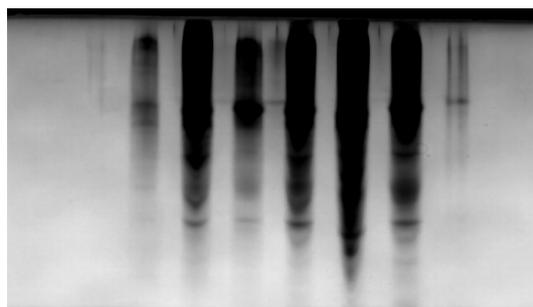


Figure 5. SDS Page gel image of vector A with added inducer

CONCLUSION

Today, due to the decreasing natural resources, microorganisms and bacteria are seen as potential in many production areas and intensive studies are carried out on this subject. As a result of the studies, many kinds of enzymes can be produced and these enzymes contribute greatly to the country's economy. 60% of these enzymes, which are an indicator of industrial development, are recombinant products. Expansin proteins are found in small amounts in plants and these proteins are not produced microbially. Expansin proteins cannot be produced in pure culture, and isolating them from plants is costly. The production of expansin proteins in recombinant protein formats would overcome these problems. In this study, a potential alternative protein to the enzymes that break down the cell wall was obtained by producing the recombinant Expansin protein. Since the expansin protein is very costly to isolate and produce, the relevant gene (LeExp 1) was produced as a recombinant protein. Since this protein obtained in the study was produced recombinantly, a new biological agent was obtained that breaks down the cell wall. Whether this protein provides a synergistic effect when used with other enzymes and economic analysis of the production for industrial applications of recombinant expansion can be evaluated in further study.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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