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VARIA

Cloning and surface expression of engineered enzyme of organophosphorus hydrolase in E. coli and evaluation of its function

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Abstract: *Nowadays, the chemical compounds of organophosphate cause biological contamination due to their presence in the environment and production of metabolites, which endanger the health of humans and organisms. Thus, it is crucial to use a safe method to eliminate the contamination of these compounds. The objective of the present study was cloning and surface expression of the engineered enzyme of organophosphorus hydrolase in E. coli and comparing its function with the non-engineered enzyme. The present study aimed at improving the function of the organophosphorus hydrolase enzyme by expressing this enzyme along with Ice nucleation protein (INP) on the surface of bacterial cells. For this purpose, the OPH enzyme coding sequence was cloned in the pET28a-InaV-N plasmid and sent into E. coli, the strain of BL21 (DE3) and protein expression was evaluated at 37° C after 16 hours of induction. To examine the site of expression of the recombinant protein, cell components (cytoplasm, inner membrane, and outer membrane) were separated and the results were analyzed by using SDS-PAGE. The results revealed that OPH enzyme protein was presented as a protein fusion with a molecular weight of 58 KDa next to the INP anchor on the surface of the outer membrane of the cell. The specific activity of the resulting protein was calculated at 3828.8 U/mg after incubation of this enzyme in the vicinity of the substrate at 37° C. based on the results of the present study, it seems that the expression of engineered enzyme and its presentation on the cell surface is an achievement in using the cell as a degrading agent of organophosphate toxins and eliminating the costs of downstream processes such as purification and refolding of protein.*

Keywords: *clone, expression, engineered enzyme of organophosphorus hydrolase, E. coli, function*

INTRODUCTION

Following developments made in a recent century in the chemical synthesis of materials, new compounds have been made, some of which are toxic. Releasing of toxic compounds into the environment such as pesticides, insecticides, solvents, explosives, and dyes in industrial and agricultural applications has increased over the last decade [1]. One of these synthetic compounds is organophosphate compounds that are used in pesticides, insecticides, and neurochemical agents. These compounds have three phosphoester bonds, so they are also called phosphotriester. Phosphorus is attached to oxygen P = O or sulfur P = S by a double bond. Paraoxon, parathion, and diazinon are considered important pesticides and insecticides made from organophosphate compounds [2]. War nerve agents include G-type and V-type. It should be noted that V agents are much more toxic than G agents. The sudden release or poor management of pesticide and insecticide residues has contaminated the soil and groundwater in the region. Also, residues of nerve agents after wars can have devastating effects on the environment

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and people. These compounds inactivate a large family of serine hydrolase enzymes (lipases and esterases) [3].

Acetylcholinesterase is one of the most important enzymes inactivated by these compounds, leading to the accumulation of acetylcholine in the nerve synapse and the continuation of nerve stimulation, and muscle dysfunction and impairment in the respiratory system respiratory failure, which results in coma and eventually death. Thus, it is essential to develop bioremediation technology to facilitate the degradation of organophosphate contaminants [4].

At present, most chemical and physical methods such as heat, superoxidase, carbon dioxide, etc. are used to remove contamination from organophosphate compounds, but they are associated with many shortcomings and disadvantages since they are often toxic, allergenic, corrosive, non-proprietary, and harmful to the environment [5]. Thus, safe technology is required to hydrolyze toxic compounds [5]. One of the useful methods for degrading the organophosphate compounds is the use of bacterial strains with enzymes that degrade these compounds with a wide substrate range.

Enzymes that as biocatalysts can degrade organophosphate compounds include organophosphorus hydrolase (OPH) derived from Pseudomonas diminuta and species of Flavobacterium, organophosphorus acid anhydrolase (OPAA) obtained from Alteromonas, and diisopropyl-phosphatase obtained from fish called Loligo vulgaris [6]. Expression of these enzymes in other hosts, including E. coli as recombinant, is one of the suitable methods for its production. OPH enzyme is present in Flavobacterium ATCC 75512 and P. diminuta.

The natural substrate for organophosphorus hydrolase is not known. This enzyme is a homodimer with a molecular weight of 72 kDa [7]. OPH can hydrolyze phosphotriester P-O bond in paraoxon, phosphonofluoride (P-F) bonds in DFP, sarin, and soman, phosphorothioate bond (P-S) in VX, and phosphoro amido cyanide (P-CN) bond in Tabun. The speed of OPH enzyme function is very high for a substrate with P-O and P-S bonds.

OPH is the only enzyme known for the degradation of both paraoxon and V-type war agents [8]. In a previous study, an engineered sample of this enzyme (creating a disulfide bridge to increase stability) was produced in the cytoplasm of E. coli. Despite changes in expression conditions, the enzyme was produced as an air inclusion and underwent difficult stages of unfolding and refolding [9].

In the present study, to solve this problem and express the enzyme in solution, by designing and synthesizing the anchor, the production of the desired enzyme was done as surface expression.

For this purpose, bioinformatics studies to design the best anchor were first considered, and then cloning, expressing, and finally measuring enzyme activity were performed.

MATERIALS AND METHODS

Preparation of materials required

Ecoli BL21 (DE3 (Novagen), pET32a (Biomatik), pET28-InaV- (Laboratory of Baqiyatallah University of Medical Sciences), LB-Agar and LB-Broth (Quelab), Yeast and Tryptone (Merck), Ampicillin, Kanamycin, Calcium chloride, XhoI, SacI (Restriction endonuclease), Agarose, DNA Safe Stain, Boric acid, Bromophenol Blue, 2-Mercaptoethanol, Taq DNA Polymerase, PFU DNA Polymerase, DNA Ladder-1Kb (# DM3200), T_4 DNA Ligase, Loading Dye, Ethanol 96%, Plasmid Extraction Kit, Fragment DNA Purification, Sodium dodecyl sulfate, Tris Base, Glycine, Glycerol, Coomassie Brilliant Blue G250, Glacial Acetic Acid, Tetramethylethylene-diamine, Isopropyl-β-thiogalactopyranoside, Acidleryed, B acrylamide Ethylenediaminetetraacetic acid, Triton X-100, Phenylmethanesulfonylfluoride, ammonium persulfate, Lysozyme, NaCl, Sucrose, and Urea (Sigma) were purchased.

pET28a-InaV-N plasmid

the pET system is one of the most powerful systems available for cloning and expression of recombinant proteins by E. coli. One of these systems is the pET-28a (+) expression vector, which includes the T7 promoter.

The gene can be cloned in the pET expression system and E. coli BL21 (DE3) can be transformed by the recombinant vector and expressed as a result of inducing by isopropyl β-D-1-thiogalactopyranoside (IPTG). pET28a includes an antibiotic marker of kanamycin. This plasmid has been used in previous studies and contains an anchor called InaV-N.

Figure 1 illustrates an image of this plasmid [10].

pET32a-OPH Plasmid

This plasmid has a T7 promoter for high protein expression and due to having a Thioredoxin tag sequence; it leads to secretion of the produced protein and has the antibiotic marker ampicillin.

This plasmid was used in a previous study conducted by Farnoush et al. The sequence of this plasmid can be seen in Appendix 1.

Figure 2 provides an image of the pET32a plasmid [11].

Figure 1: Plasmid pET28

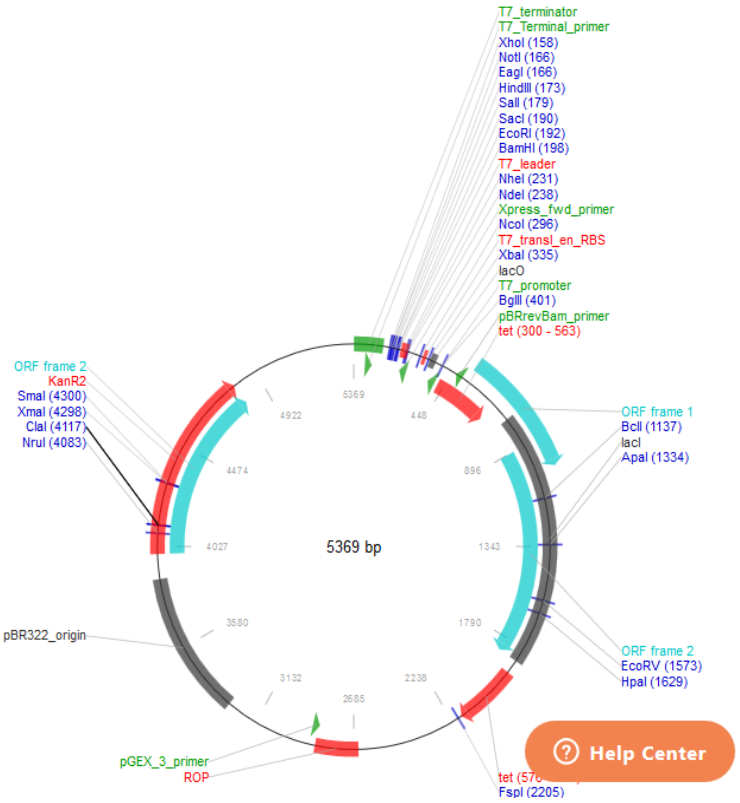
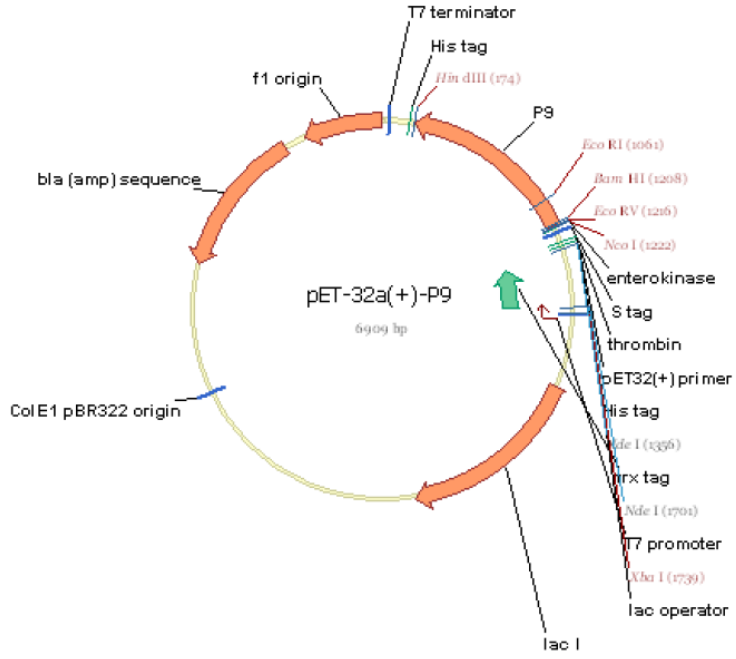


Figure 2: Plasmid image containing OPH gene, The OPH gene is cloned between the two enzyme sites of BamHI and HindIII



Plasmid purification

Plasmid purification was performed from strains containing plasmid pET28a and pET32a based on the standard protocol of the kit manufacturer. In short, 3 ml of the 18-hour culture of the strains was centrifuged and the supernatant medium culture was completely removed. The precipitate of cells was dissolved in 200 µl of FAPD1 buffer, 200 µl of FAPD2 buffer,

and 300 µl of FAPD3 buffer and centrifuged at 250,000 rpm for 5 minutes. The transparent supernatant was poured on the columns for the kit and the columns were washed twice with 400 and 700 µl of W1 and Wash buffer and 50 µl of EB buffer was poured on each column and the desired plasmids were collected by centrifugation after 5 minutes [12].

Evaluation of purified plasmids by observing on agarose gel

Agarose gel electrophoresis is a standard method for isolating and identifying DNA fragments. A certain percentage of agarose is dissolved in the TBE buffer and, when cooled, forms a substrate that acts as a molecular sieve to separate DNA fragments based on their size in an electric field.

Polymerase chain reaction

For OPH gene amplification, two direct and inverse primers were required. These two primers were designed based on the initial and final sequences of the OPH gene. The direct primer is the complement at the beginning of the gene and the reverse primer is the reverse complement of the end of the gene. To insert this gene into the pET28a plasmid between SacI and XhoI sites, the cutting site of these enzymes was designed on direct and inverse primers and the synthesis order was given to the desired company (Table 1) [13].

Table 1: Primers of gene encoding OPH enzyme

Primer name	Primer sequence
Forward (p263)	5'_ CATGAGCTCTCTATCGGCACTGGTGAC_3'
Reverse (p264)	5'_ CATCTCGAGGCTTGCACGCAGAGTC_3'

Amplification of gene encoding OPH

To amplify the gene encoding OPH enzyme, the polymerase chain reaction was performed using designed primers according to the values presented in Table 2. PCR solution was prepared in 0.2 ml microtubes. In this reaction, the used model is pET32a, which contains the OPH gene, and a negative control sample was prepared according to previous values without the pET32a plasmid.

The microtubes were placed inside a thermocycler and reaction was done according to the temperature program presented in Tables 3-5 (Table 2) [14].

Confirming plasmid transfer into bacteria

To confirm the process of transfer of recombinant pET28-InaV-N/OPH plasmid into bacteria, PCR was performed for colonies grown on a solid culture medium. For each colony, some amounts were inoculated in 30 µl of sterile distilled water. Then, 15 µl of it was taken in 1 ml of liquid culture medium.

The remaining 15 µl was placed at 94° C for 15 minutes and then centrifuged at 13000 rpm for 2 minutes so that the broken cells precipitate. Supernatant media were used as a template in the PCR reaction.

The PCR reaction was prepared according to the values presented in Table 3 and performed according to the program of Table 4 [15].

Table 2: Values used in the PCR reaction to amplify the OPH gene

Material	Amount (µl)
Template (1 µg/µl)	10
Forward Primer (10µM)	2.5
Reverse Primer (10µM)	2.5
PFU SuperMasterMix 2x	25
Sterile deionized water	10
Total volume	50

Table 3: Values used in the PCR reaction for amplification of OPH gene

Material	Amount (µl)
Template (1 µg/µl)	2
Forward Primer (10µM)	0.25
Reverse Primer (10µM)	0.25
Taq 2x Master Mix RED	2.5
Total volume	5

Table 4: Values used in the PCR reaction to amplify the OPH gene

Cycle	Temperature (°C)	Time	Number of cycles
Primary denaturation	94	5 min	1
Denaturation Primer connection To lengthen	94 55 72	45 s 60 s 45 s	35
The final elongation	72	5 min	1

Enzymatic digestion

For cloning the OPH gene within the pET28a plasmid, both the amplified gene and the purified plasmid need to be cut using similar enzymes. It causes a complementary adhesive end on the gene and the plasmid and facilitates the binding of the gene to the plasmid.

Cutting both sides of the amplified gene was done using XhoI and SacI enzymes according to the values presented in Table 5.

The mentioned values were poured into 0.5 ml microtubes and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme [16].

Table 5: Values used for enzymatic digestion of gene amplified by PCR

Material	Amount (µl)
The amplified gene	50
Enzyme 10 Unit/µl XhoI	2
Enzyme 10 Unit/µl SacI	2
Universal buffer (10X)	7
Sterile water	9
Total volume	70

Cutting of plasmid pET28a using two enzymes of XhoI and SacI

The plasmid was cut according to the values of Table 3-10 by two enzymes of XhoI and SacI to linearize the pET28a-InaV-N plasmid and remove the previous fragment. The mentioned values were poured into a 0.5 ml microtube and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme (Table 6) [17].

Table 6: Values used in enzymatic digestion of plasmid pET28a

Material	Amount (µl)
Plasmid pET28a-InaV-N/OPH	5
Enzyme 10 Unit/µl XhoI	0.25
Enzyme 10 Unit/µl Sac I	0.25
Universal buffer (10X)	1
Sterile water	3.5
Total volume	10

Confirming entry of OPH gene into plasmid pET28-InaV-N by enzymatic digestion

To confirm the performed process and ensure the presence of the gene in the plasmid pET28-InaV-N, the plasmid was cut using two enzymes of XhoI and SacI with the values listed in Table 7. The mentioned values were poured into a 0.5 ml microtube and placed in a thermoblock for 20 minutes at 37° C. Then, the microtube was incubated for 15 minutes at 65° C to inactivate the enzyme [18].

Table 7: Values used in enzymatic digestion of OPH

Material	Amount (µl)
Plasmid pET28a-InaV-N/OPH	5
Enzyme 10 Unit/µl XhoI	0.25
Enzyme 10 Unit/µl Sac I	0.25
Universal buffer (10X)	1
Sterile water	3.5
Total volume	10

Extraction of cut fragments from agarose gel using Kiagen kit

After cutting the gene and plasmid, it is necessary to separate these fragments from other extra fragments in the solution and then bind them together in a binding reaction. For this purpose, the product obtained from enzymatic digestion of plasmid was first taken on agarose gel and then extracted from the gel according to the kit protocol. The cut gene fragment was separated from the small cut fragments with the same kit without being placed on the gel. The gene was then purified by the clean-up method [19].

OPH enzyme protein expression

Plasmid gene-binding product was transferred into susceptible bacterial cells by the electroporation method. The culture was performed from colonies containing OPH gene plasmid in 5 ml of 2XY culture medium. When the optical absorption of the culture medium at 600 nm reached 0.7, after removing 1 ml of culture medium as an induction-free control, the promoter was induced by adding IPTG with a final concentration of 1 mM, and with the control sample, it was placed in an incubator shaker at 37° C for 18 hours. After the mentioned time, 1 ml of induced and control bacterial culture was precipitated and after adding 60 µl of 8 M urea, 16 µl of them were combined with 4 µl of sample buffer and heated for 5 minutes loaded on polyacrylamide gel [20, 21].

Primary evaluation of enzyme activity expressed on the surface

To evaluate the activity of the enzyme present on the surface, a precipitate of enzyme-expressing cells was collected and after twice washing with Tris buffer, 980 µl of cells with OD = 1 was poured into the microtube and 10 µl of cobalt chloride ion was added and 10 µl of parathion toxin was incubated for 1 h at 37° C [22].

Confirming surface expression of the enzyme

To locate the protein expression in the recombinant strain, it is necessary to first separate the cell components and then examine the fractions.

For this purpose, 20 ml of an overnight culture of control and test samples after protein expression, and their centrifugation and precipitate were dissolved in the lubricating buffer after washing twice in Tris buffer, and after half an hour of incubation at room temperature, the cell wall was broken with the sonicator device (4 30-second cycles with 80 power) was broken.

Then, centrifugation was performed at 2000 rpm for 3 minutes to separate the unbroken cells, and their

supernatant was re-centrifuged at 14000 rpm for one hour. After emptying the supernatant into separate falcons, the precipitates were dissolved in Tris buffer containing Triton X-100 and 0.01 mM $MgCl_2$ and incubated at ambient temperature for 30 minutes to separate the inner and outer membranes.

Then, centrifugation was n performed at 13000 rpm for one hour. At this stage, the supernatant contains the inner membrane and the precipitate contains the outer membrane and the proteins attached to it. 500 µl of Tris buffer was added to both precipitates and dissolved.

Sampling was performed from each stage and examined using SDS-PAGE [23].

Evaluation of the specific activity of the enzyme in cell fractions

To evaluate the enzyme activity of the expressed OPH protein, a 50 mM Tris buffer was prepared.

The reaction medium containing 970 µl of Tris buffer, 20 µl of the sample (cytoplasmic samples, inner membrane, and outer membrane), 10 µl of cobalt ion with a concentration of 0.1 mM and 10 µl of parathion toxin with a concentration of 5 mg/ml were prepared.

After incubation at 37° C, the optical absorption of the samples was read at 405 nm and the specific activity of the enzyme was calculated using the following formula [24]:

$$\text{U/ml enzyme activity} = \frac{\text{dilution coefficient} \times \text{reaction volume} \times \text{absorption at 405nm}}{\text{time} \times \text{enzyme value} \times \text{PNP degradation coefficient}} \times 10^6$$

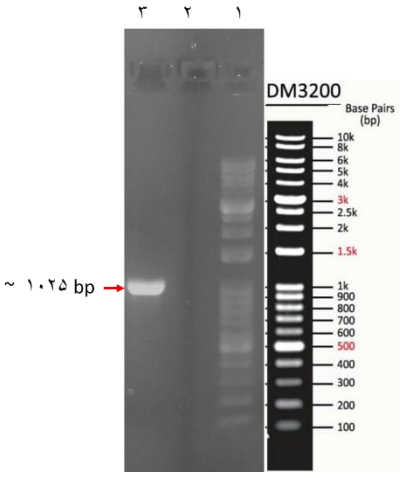
Reaction volume: 1 ml; Reaction time: 60 minutes; Dilution coefficient: 1; PNP degradation coefficient: 18000
Enzyme specific activity (U/mg) = normal activity (U/ml) / enzyme value (mg)

RESULTS

Amplification of OPH gene using PCR

In the first step, the OPH gene was amplified using p263 and p264 primers, and the PCR product was placed on agarose gel for confirmation. An observing band with a size of 1025 bp confirmed the amplification of the OPH gene. The OPH enzyme encoding gene is a 1008 bp sequence that, along with an enzyme site designed on primers, shows a length of about 1025 bp on the agarose gel (Figure 3).

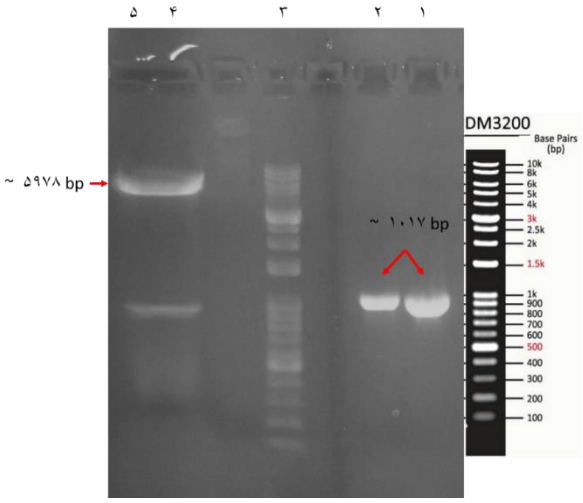
Figure 3: OPH gene amplified by PCR.1- Marker # DM3200, DNA 1kb. 2. Negative control. 2. OPH amplified gene



Evaluation of fragments obtained from PET28a-InaV-N plasmid cutting and PCR product on agarose gel

Plasmid pET28a-InaV-N and OPH gene PCR products were cut using XhoI and SacI enzymes and some of each reaction was loaded on an agarose gel to confirm the enzymatic digestion process. The 1017 bp band observed on the gel displaying the OPH gene and the 5978 bp band relate to the cut plasmid pET-InaV-N (Figure 4).

Figure 4: Fragments obtained from enzyme cutting of plasmid pET28a-InaV-N and PCR product. 1 and 2-fragments obtained from cutting OPH gene amplified by PCR.3-marker # DM3200, DNA 1kb. 4,5-plasmid pET28a- cut InaV-N

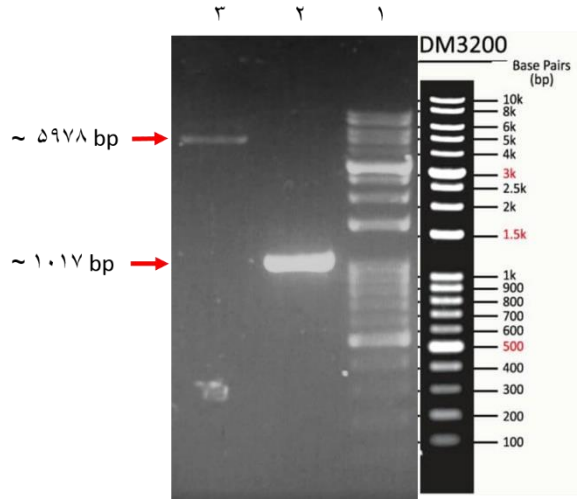


Extraction of plasmid and gene from agarose gel using Kiagen kit

After performing the enzymatic digestion process and examining the accuracy of cuts made with agarose gel, the area related to the desired fragment was cut and removed from the gel with the help of a special kit. The results of this purification were evaluated on a gel (Figure 5) and its

concentration was measured with a nanodrop. The concentration of purified gene and plasmid was determined to be 144 ng and 82 ng, respectively.

Figure 5: Extraction of cut fragments from agarose gel. 1-marker # DM3200, DNA 1kb. 2. cut OPH gene

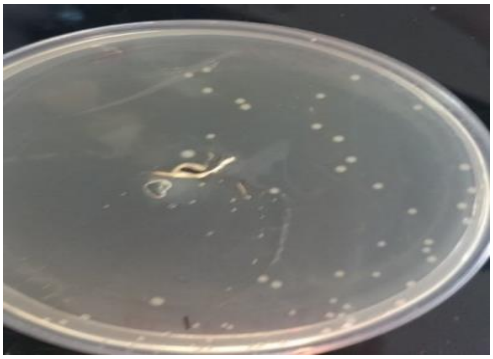


Transfer of recombinant pET28a-InaV-N / OPH plasmid into E.coli BL21 strain (DE3)

After proximity of the OPH gene and pET28a-InaV-N at the presence of the enzyme ligase, the recombinant plasmid was transferred into a bacterium by electroporation E. coli BL21 (DE3) and cultured on an agar medium containing the antibiotic kanamycin.

As shown in Figure 6, plasmid-containing colonies grew overnight.

Figure 6: Colonies obtained from pET28a-InaV-N / OPH transforming into E. coli BL21 strain (DE3).

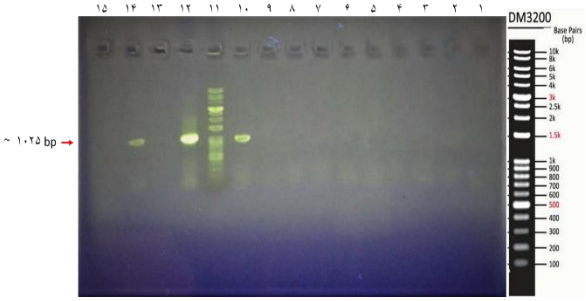


Confirming of colonies containing recombinant plasmid pET28a-InaV-N/OPH by direct PCR method from colonies

To evaluate the accuracy of transfer of the recombinant plasmid within the bacterial strain, 12 colonies grown on the medium were used for PCR. Among the tested colonies, the 1025 bp band, which is amplified by the OPH gene, was observed only in colonies 10 and 14.

The gene-free sample was considered as negative control and the sample containing plasmid pET32a-OPH was considered as a positive control (Figure 7).

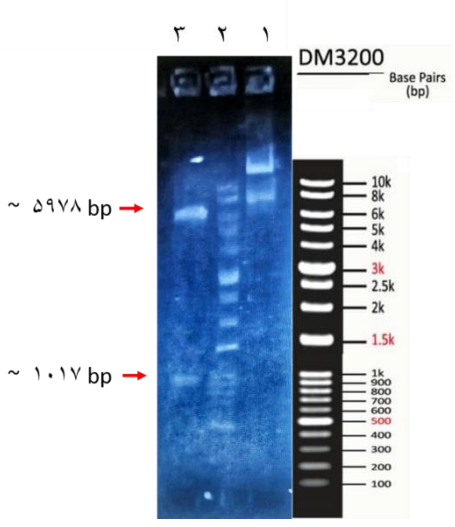
Figure 7: Results of direct PCR of colonies grown on agar. Samples 1-10, 14, and 15 of PCR products of colonies. 11- DNA marker 1kb # DM3200. 12-Positive control. 13-Negative control



Two-enzyme digestion of plasmid pET28a-InaV-N/OPH

The final confirmation of the presence of recombinant plasmids in BL21 strain (DE3) was performed by digestion with two enzymes of XhoI and SacI. The 1017 bp band obtained from the plasmid cut in Figure 8 indicates the presence of the gene.

Figure 8: Two-enzyme digestion of plasmid pET28a-InaV-N / OPH. 1. Uncut plasmid. 2-DNA1kb marker. 3. Cut plasmid.

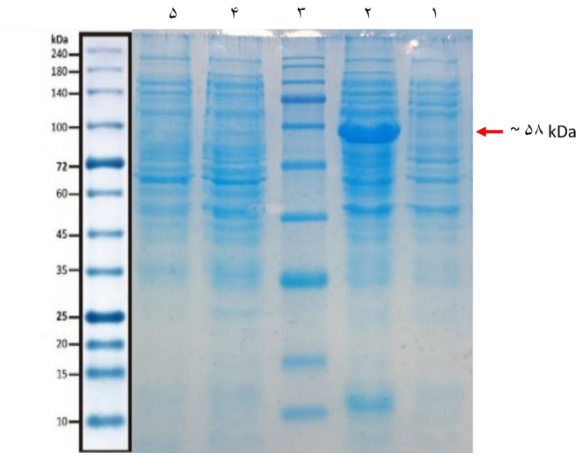


Induction of expression for two confirmed colonies

Two confirmed colonies were inoculated in 5 ml of culture medium and expression was induced after reaching OD600 of culture medium to 0.7. The results obtained on polyacrylamide gel are as follows. As shown in Figure 9, the

58 kDa band is observed only in one of the test samples and is not observed in-band control sample.

Figure 9: Expression of OPH enzyme in BL21 strain (DE3) at 37° C. 1 and 5 - samples of the negative control. 2 and 4 - Induced samples after 5 hours of incubation at the presence of IPTG inductor. 3 - Protein marker # PM1700



Evaluation of enzyme activity expressed on the surface

To evaluate the activity of the enzyme present on the surface at 37° C, 18 hours after adding IPTG, the precipitate of cells of test and control samples was collected and entered into the reaction medium. After one hour, the absorption of the reaction medium was read at 405 wavelengths and the enzyme activity was calculated. The samples include bacteria without the OPH gene and cytoplasmic enzyme-producing bacteria. As shown in Table 8, the activity of the enzyme expressed at the surface is lower than that of the enzyme produced as a solution in the cytoplasm.

Table 8: Comparison of enzyme activity expressed on the surface and sample expressed in the cytoplasm

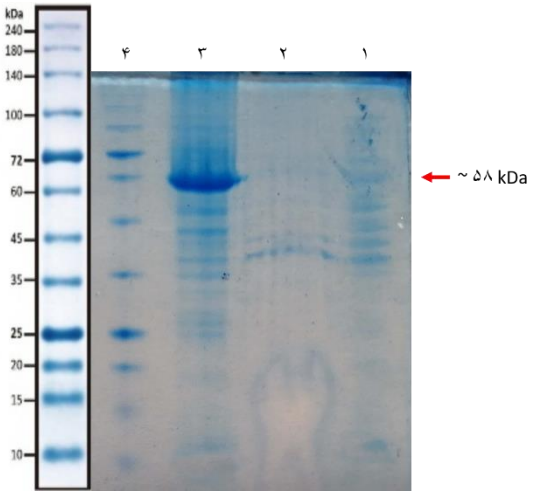
Enzyme Activity (U/ml)	37^0 C
Gene without genes	0.74
Cytoplasmic expression	2.77
Superficial expression	7.67

Evaluation of the specific activity of OPH enzyme

After separating the cell components, the fractions related to each fraction of the cell were first examined by SDS-PAGE. Then, their activity was measured based on mentioned conditions, and the obtained data are shown in Figure 10. As shown in the figure, most of the activity is related to the outer membrane. It means the expression of enzymes on the surface of the outer membrane of the bacterium.

The specific activity of the OPH enzyme was estimated at 3828.8 U/mg using the formula mentioned in Figure 10 and Table 9.

Figure 10: Separation of cell fractions after protein expression at 37° C



2. Cytoplasmic fraction. 2. Inner membrane fraction. 3. Outer membrane fraction. 4-Protein marker # PM1700

Table 9: Examining the enzymatic activity of each cell fraction

The activity of each cell segment (U/ml)	37^0 C
Cytoplasmic section	3.6936
Internal membrane section	1.2162
Outer membrane section	7.6576

DISCUSSIONS

Organophosphate compounds, including pesticides and neurochemicals, are very dangerous and insoluble in aqueous systems. Despite their high toxicity, organophosphate pesticides are still widely used to protect plants in agriculture. Due to the accumulation of these compounds in food products and water resources and its irreversible effects on human health and other organisms, it is essential to develop an advanced, appropriate, and cost-effective method to eliminate these compounds from the environment. Among the various physical, chemical, and biological methods, the use of natural strains as biodegraders has high importance. Strains such as Pseudomonas diminuta MG and Flavobacterium sp. ATCC 21881, which uses organophosphate molecules as food sources, can degrade a wide range of these compounds, including pesticides and neurochemical agents [25]. The degrading enzyme identified in these strains, called organophosphorus hydrolase (OPH), contains 336 amino acids and has a molecular weight of 36 kDa. The gene encoding this enzyme was used for expression in the cytoplasmic space of BL21 (DE3) bacterium after two mutations at sites 99 and 124 and altering two amino acids

of threonine and glutamic acid to cysteine to create a disulfide bond, but its expression was mainly accumulated as protein masses in the cytoplasm and was dysfunctional and without proper structure [26, 27]. Thus, the bacterial cell must first be lysed and after extracting the accumulated proteins, they must be solubilized with a strong denaturator and the refolding process must be continued in the presence of appropriate stabilizers. All of these steps require much time and money, and finally, with the loss of a part of the protein, a protein is obtained that often does not regain its structure 100% and does not have good stability [28]. Another form of OPH enzyme expression is performed as periplasmic expression. In research carried out in 2015 by Latifi et al, the OPH enzyme encoding sequence in E. coli was synthesized and the OPH enzyme was expressed in the form of air inclusion using pET21a-opd and pET26b-opd in periplasmic space. Periplasmic expression of this enzyme also leads to limited production of this enzyme and requires complementary processes such as bacterial cell breakdown and protein extraction from the cell [25]. Besides the various methods proposed for the expression of recombinant proteins as a solution, including reducing the temperature, reducing the concentration of inducer, and using sequences that help solubilize the protein, the use of membrane anchors to provide protein on the bacterial cell surface is one of the most effective strategies used to express a wide range of proteins and has yielded promising results since molecule when attached to a matrix-like the cell surface is more accessible and much more stable than the free molecule and does not require long and costly purification steps [26, 27]. The use of natural integral proteins of the outer membrane as carriers is necessary for the goals of presentation on the surface. A variety of surface anchor motifs such as Lpp-OmpA, INP, and auto-transporters are used in this system due to their properties [31]. OPH enzyme expression in E.coli has been performed as a surface expression by various researchers. For example, in a study conducted by Richins et al in 1997, OPH enzyme expressed the OPH protein on the surface of E. coli using an Lpp-OmpA anchor system [32]. Shimazu et al in 2001 examined the expression of OPH protein by three anchors, including Inak-NC, InaV-NC, and Lpp-OmpA. The Inak anchor motif was derived from P. syringae KCTC 1832 and the InaV motif was derived from P. syringae INA [33]. Dong Kang et al in 2008 used only the second InaK amine to provide OPH on the surface [34]. In each of these studies, the OPH enzyme was expressed by different anchors on the cell surface. As a surface expression of OPH enzyme in previous studies in E. coli has resulted in the production of functional and highly stable proteins, and since the engineered OPH enzyme used in this study has been previously expressed in the cytoplasm due to its disulfide bond in the air inclusion and non-functional form, this sequence was re-expressed differently in E. coli in the form of surface expression using the InaV-N anchor. The results of investigating the activity of different parts of cell fractions showed that the fraction related to the outer membrane has the highest activity and has an activity more than twice as much as other cell fractions. Inav-N anchor not only can transfer OPH protein on the surface of bacteria but also has a positive effect on its activity.

CONCLUSIONS

The main objective of the present study was a surface expression and improving the efficiency of the engineered enzyme OPH (containing disulfide bridge) which was expressed in the previous study as air inclusion. The use of InaV-N anchors, which is one of the known anchors for surface expression, responded well to the desired enzyme and the enzyme could maintain its activity after surface expression. In a conclusion, this study could solve the problem of the insolubility of the protein, maintain its structure and its desirable activity, while surface expressing the engineered protein using the InaV-N anchor on E. coli bacteria.

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