Research in Molecular Medicine

Design and Production of Sal k 1 Antigens for Allergy Diagnosis in Humans

Nastaran Farahani[1], Shohreh Zare Karizi[1*]

1. Department of Genetic and Biotechnology, Faculty of Biological Sciences, Varamin Pishva Branch, Islamic Azad University, Varamin, Iran.

Use your device to scan and read the article online

Citation Farahani N, Zare Karizi Sh. Design and Production of Sal k 1 Antigens for Allergy Diagnosis in Humans. Research in Molecular Medicine. 2024; 12(2):81-90. https://doi.org/10.32598/rmm.12.2.1363.1

https://doi.org/10.32598/rmm.12.2.1363.1

Article Type:
Research Paper

Article info:
Received: 22 Aug 2023
Revised: 02 Jan 2024
Accepted: 28 Feb 2024

Keywords:
Allergy, Diagnosis, Sal k 1, *Salsola Kali*

ABSTRACT

Background: Allergy is highly prevalent in different parts of the world. One of the important allergens is Sal k 1 protein (from *Salsola Kali*, which is a member of the weed family). The present study aimed to design an effective Sal k 1 recombinant protein for diagnostic purposes.

Materials and Methods: In this study, the Sal k 1 gene was expressed in *Escherichia coli* BL21 (DE3) cells following induction with IPTG. SDS-PAGE analysis revealed a distinct protein band at 51.7 kDa. Protein purification was performed using a Ni-NTA combined molecular chromatography column under natural conditions. Protein expression and diagnostic potential were subsequently confirmed through Western blot analysis and enzyme linked immunosorbent assay (ELISA).

Results: To examine the expression at different hours after induction with IPTG, significant expression was observed using SDS-PAGE at 16 hours. The amount of recombinant protein in denatured conditions was more appropriate than in native conditions.

Conclusion: In conclusion, the successful production of recombinant Sal k 1 protein may be useful in allergy diagnostics.

*** Corresponding Author:**
Shohreh Zare Karizi, PhD.
Address: *Department of Genetic and Biotechnology, Faculty of Biological Sciences, Varamin Pishva Branch, Islamic Azad University, Varamin, Iran.*
Phone: *+98 (912) 5953602*
E-mail: *shohrehzare@yahoo.com*

Introduction

Nowadays, the occurrence of allergic diseases has increased in the world. Allergy is known as an overreaction of the immune system to a foreign substance, such as pollen, bee venom, pet dander, or food, which are referred to as allergens [1]. Individuals with allergies have an overactive immune system. The severity of allergies varies, ranging from mild irritation to anaphylaxis [2]. The most common cause of allergies worldwide is pollen from trees, grasses, and weeds. Typically, an allergic reaction can occur anywhere in the body where immune cells are located [3]. The body's immune cells attack allergens that come into contact with the skin, or enter the body through inhalation, ingestion, or injection, causing allergy symptoms [4]. Inflammation plays a fundamental role in the pathophysiology of asthma. Airway inflammation is associated with the response of various immune cells and mediators, which ultimately leads to changes in the pathophysiology of asthma. Bronchial inflammation and airway obstruction lead to clinical manifestations of cough, wheezing, and shortness of breath [5, 6]. Factors that contribute to the development of asthma include innate immunity, genetics, gender, and the individual's cytokine pattern. Environmental factors, such as airborne allergens and respiratory infectious viruses, are considered the most important factors in the development, resistance, and severity of asthma, along with age [7].

The two main classes of asthma therapy are long-term controller therapies (including corticosteroids, long-acting beta-agonists (LABA), leukotriene modifiers (LTRA), cromolyn, nedocromil, and methylxanthines) and rapid-relief therapies (including short-acting bronchodilators, systemic corticosteroids, and anticholinergics) [8]. Recently, allergen-specific immunotherapy (AIT) based on natural allergen sources has emerged as a helpful therapeutic approach for allergies related to common allergens such as grass pollen and house dust mites [9]. Besides, recombinant allergens are increasingly used for diagnostic and therapeutic purposes.

Sal k 1 is the first allergen identified from *Salsola kali*. It is a polymorphic protein with a molecular weight of approximately 37 kDa and also has more than 20 isoforms with different isoelectric points ranging from Pl 4 to 9.5; it belongs to the pectin methylesterase protein family [10, 11]. It is responsible for more than 80% of allergic and sensitizing cases. Sal k 1 is considered a marker in the identification of allergic agents, especially related to *S. kali* pollen, since Sal k 1 itself is a major reason for the differences in sensitivities to *S. kali* pollen and *Chenopodium album* pollen [12]. The expression of the Sal k 1 protein was reported in 2010. Recently, Sal k 1 has also been produced in bacteria and yeast, showing a slight involvement of the glycan component in sensitivity to Sal k 1. The researchers have also demonstrated that recombinant Sal k 1 has the potential to be used in clinical diagnostics, as it contains all the immunological properties of most isoforms present in the wild-type protein [13, 14]. Therefore, the aim of the present study was to produce and express recombinant Sal k 1 protein in the expression host *Escherichia coli* as a means to diagnose allergies and examine the intensity of IgE binding to Sal k 1.

Materials and Methods

Transfection of the pUC57 vector containing the *Sal k 1* gene into the TOP10 strain

The standard calcium chloride method was used to prepare susceptible *E. coli* TOP10 and *E. coli* BL21 (DE3) cells. Two vectors were used to optimize expression: pET-28a (+), chosen for its strong T7 promoter and kanamycin resistance, ideal for initial solubility screening, and PUC-57. Also, we used a tiered approach that ensured rigorous validation. In this regard, we used TOP10 to guarantee plasmid integrity, BL21 (DE3) to establish an expression baseline, and Rosetta (DE3) to resolve codon bias issues identified in BL21. The *Sal k 1* gene sequence was delivered after ordering in the PUC-57 vector. The nucleotide sequence of the gene is presented in Table 1. Transformation was performed by the cold-heat shock method. Under sterile conditions, 5 µL of plasmid pUC57 was added to 100 µL of TOP10 susceptible cells. To purify the PET-SUMO plasmid, alkaline lysis was used according to the plasmid extraction protocol of the Genentech Bio's extraction kit.

Sal k 1 gene amplification by PCR reaction

A pair of forward and reverse primers was designed to amplify the *Sal k 1* gene. The restriction enzyme cleavage site sequences, SspI and XhoI, were incorporated into the primers. The primer sequences are presented in Table 2. The PCR reaction was performed using the primer pairs in a total volume of 25 µL (2.5 µL PCR buffer, 0.5 µL dNTPs (50 mM), 1 µL $MgCl_2$ (1.5 mM), 1 µL forward primer, 1 µL reverse primer, 100 ng template DNA, 1 unit Taq polymerase, and 18.5 µL distilled water) with a temperature gradient of 55-62 ˚C. Moreover, to amplify the desired gene, the PCR reaction was performed using specific primers and the Pfu enzyme,

Table 1. The nucleotide sequence of *Sal k 1* gene

Name	Number of Amino Acids	Sequence
Sal K 1	1017	CAACTTATTCCGCCGAATCCGGCAGAACTTGAATCATGGTTTCAAGGCGCAGTTAAACCGGTTTCAGAACAAAAAGGCCTTGAACCGTCAGTTGTTCAAACAGAATCAGGCGGCGTTGAAACAATTGAAGTTAGACAAGATGGCTCAGGCAAATTTAAAACAATTTCAGATGCAGTTAAACATGTTAAAGTTGGCAATACAAAAAGAGTTATTATTACAATTGGCCCGGGCGAATATAGAGAAAAAGTTAAAATTGAAAGACTTCATCCGTATATTACACTTTATGGCATTGATCCGAAAAATAGACCGACAATTACATTTGCAGGCACAGCAGCAGAATTTGGCACAGTTGATTCAGCAACAGTTATTGTTGAATCAGATTATTCAGTTGGCGCACATCTTATTGTTACAAATTCAGCACCGAGACCGGATGGCAAAAGAAAAGGCGCACAAGCAGGCGCACTTAGAATTTCAGGCGATAGAGCAGCATTTTATAATTGCAAATTTACAGGCTTTCAAGATACAGTTTGCGATGATAAAGGCAATCATTTTTTTACAGATTGCTATACAGAAGGCACAGTTGATTTTATTTTTGGCGAAGCAAGATCACTTTATCTTAATACAGAACTTCATGTTGTTCCGGGCGATCCGATGGCAATGATTACAGCACATGCAAGAAAAAATGCAGATGGCGTTGGCGGCTATTCATTTGTTCATTGCAAAGTTACAGGCACAGGCGGCACAGCACTTCTTGGCAGAGCATGGTTTGATGCAGCAAGAGTTGTTTTTTCATATTGCAATCTTTCAGATGCAGCAAAACCGGAAGGCTGGTCAGATAATAATAAACCGGAAGCACAAAAAACAATTCTTTTTGGCGAATATAAAAATACAGGCCCGGGCGCAGCACCGGATAAAAGAGCACCGTATACAAAACAACTTACAGAAGCAGATGCAAAAACATTTACATCACTTGAATATATTGAAGCAGCAAAATGGCTTCTTCCGCCGCCGAAAGTT

which has proofreading properties. The DNA template used was the purified PET-SUMO plasmid carrying the *Sal K 1* gene.

Enzymatic digestion of the pET-SUMO plasmid and PCR product

The PCR product of the PET-SUMO plasmid containing the *Sal k 1* gene was digested with two enzymes, SspI and XhoI, for 2 hours according to Table 2 at 37 ˚C. The digestion products were run on a 1% agarose gel for confirmation. The ligation reaction of the target fragment with the plasmid was carried out using T4 ligase enzyme for 2 hours at 22 ˚C (T4 ligase buffer (4 µL), *Sal k 1* gene (15 µL), pET-SUMO (10 µL), T4 ligase (2 µL). Then, the recombinant vector (pET-SUMO with *SAL k 1* gene) was transferred to susceptible BL21 and ROSETTA cells, both of which were expressing, using the cold-heat shock method. To check the accuracy of sub-cloning, colonies grown on solid LB plates containing kanamycin (concentration 50 µg/mL) were first used to perform PCR colony analysis.

Expression of the recombinant *Sal k 1* gene

In order to investigate the *Sal k 1* gene expression in the pET-SUMO plasmid in both BL21 and ROSETTA hosts, eight colonies were cultured in liquid LB media containing 50 µg/mL kanamycin for 18 hours in a shaker incubator at 37 ˚C and 180 rpm. Then, 50 µL of each culture was added separately to 5 mL of LB media containing 50 µg/mL kanamycin. After the turbidity of bacterial growth at a wavelength of 600 nm reached 0.6-0.8, 1 mL of each colony was separated and precipitated as a control. Then, IPTG was added to the culture medium at a final concentration of 1 mM under sterile conditions and kept in a shaker incubator for 4 and 24 hours at 37 ˚C and 180 rpm. Then, 1 mL of each test tube and control tube was poured into 1.5 mL micro-tubes, and the cells were collected by centrifugation for 1 minute. The collected cells were directly mixed with sample buffer (1X) and, after boiling for 10 minutes, electrophoresed via SDS-PAGE.

Confirmation of recombinant Sal k 1 protein by Western blotting

The transgenic bacterial colonies containing the recombinant plasmid were electrophoresed on a 10% SDS-PAGE gel before and after induction of selection and expression of the desired protein. After separation of the protein bands in the sample on the SDS-PAGE gel, the gel was removed from the two glass plates for Western blotting. Briefly, after staining, the membrane was immersed in blocking buffer (5 g of skim milk in 100 mL TBS (1X)) for 1 hour at 37 ˚C and then washed with TBS (1X)/Tween 20%. Anti-His tag antibody was prepared at a ratio of 1:2000 in TBST (1x) buffer and was poured onto the PVDF membrane and placed in a shaker incu-

Table 2. Sequences of the primers

Gene		Primer Sequence	Product Length (bp)
Sal K 1	Forward	CCCCCCAATATTCAACTTATTCCGCCGAATCC (SspI)	1017
	Reverse	ATATATCTCGAGTTAAACTTTCGGCGGCGGAAGAA (XhoI)	

bator for one hour at 37 ˚C. Then, it was washed three times with TBS buffer for 10 minutes each time.

Determination of Sal k 1 protein activity by enzyme linked immunosorbent assay (ELISA)

In this experiment, the indirect ELISA method was used to measure the functional level of the Sal k 1 protein. According to the references, the functional level of the recombinant Sal k 1 protein can be measured using the serum of individuals allergic to this protein and evaluating the interaction between the recombinant Sal k 1 protein and human anti-IgE in the ELISA method.

In this study, ten serum samples were examined in the ELISA test (7 samples with sensitivity to Sal k 1 protein and three serum samples from individuals without any history of hypersensitivity). Patient samples were selected based on confirmation from clinical specialists. In summary, the microplate was first coated with 1 µg of purified and dialyzed recombinant Sal k 1 protein. To control the components of the ELISA reaction, two wells were designated as controls (one without protein and one without antibody) in the relevant column. Also, one well was considered a positive control with commercial Sal k 1 protein (1 µg). The antigen-free areas on the bottom of the wells were then blocked with PBST buffer containing 3% nonfat dry milk. Furthermore, anti-human IgE antibody was diluted at a ratio of 1:2000 in PBST buffer and added to each well, and placed at 37 ˚C for 1 hour. Subsequently, an appropriate dilution (1:5000) of human conjugated antibodies (anti IgE- HRP conjugate) was prepared in PBST buffer, and 100 µL of this solution was added to each well. Finally, the OPD substrate tablet (Sigma) was completely dissolved in 20 mL of citrate phosphate buffer, and then, 50 µL of H_2O_2 was added to it. Next, 100 µL of this solution was added to each well, and the microplate was transferred to a dark place to perform the enzymatic reaction. After the solution changed color to yellow, the reaction was stopped with 2 M sulfuric acid. Then, the absorbance was read at 492 nm.

Results

Purification of the PUC57 plasmid

The PUC57 cloning vector was transformed into susceptible TOP10 bacteria, and the clones were screened on LB agar medium containing ampicillin and kanamycin. Figure 1 shows the results of purification of the 2710 bp plasmid PUC57 on a 1% agarose gel.

Amplification of the *Sal k 1* gene using PCR and Pfu enzyme, along with enzymatic digestion of the pET-Sumo plasmid and the PCR product

For gene amplification in the pET-Sumo plasmid, a temperature gradient of 55 to 62 ˚C was established. The optimum temperature was chosen to be 59 ˚C with 1.5 mM $MgCl_2$ (Figure 2a). In order to amplify the *Sal k 1* gene, a PCR reaction was performed using its specific primers and the Pfu enzyme. According to Figure 2b, the *Sal k 1* gene produced a 1017 bp band after amplification with Pfu. According to Figure 2c, the PCR product (*Sal k 1*) and the pET-Sumo plasmid were digested with two enzymes, SspI and XhoI, for cloning, and their enzymatic digestion products were electrophoresed on a 1% agarose gel.

Transformation, screening of clones containing recombinant DNA, and confirmatory digestion of the extracted plasmid with XhoI and SspI enzymes

The ligation reaction was performed using the vector and the PCR product. The ligation product was transformed into susceptible cells in the BL21 host by the heat shock method and transferred onto LB agar medium containing kanamycin. To confirm the cloning, 4 colonies were used for gene amplification (*Sal k 1*) (Figure 3a). To confirm the clones obtained, the extracted plasmids were first digested with the two enzymes XhoI and SspI that were used for cloning. The enzymatic digestion product was electrophoresed again on a 1% agarose gel, and the results are shown as the PET-SUMO band and the *Sal k 1* gene in Figure 3b.

Transfer of the recombinant plasmid to susceptible BL21 and Rosetta cells and examination of its expression with SDS-PAGE

After extracting the recombinant plasmid from the TOP10 host, it was transfected into susceptible BL21 *E. coli* cells. To verify the transformation of the recombinant vector, PCR colony analysis was performed. Figure 4a shows the results of PCR colony analysis of transformed BL21 colonies. At this stage, after culturing the bacteria and inducing them with IPTG, gene expression was performed, and the results were examined on a 10% SDS-PAGE gel. The expression results are shown in Figures 4b and 4c. The SAL K 1 protein with a band of 51.7 kDa is visible in both BL21 and ROSETTA hosts.

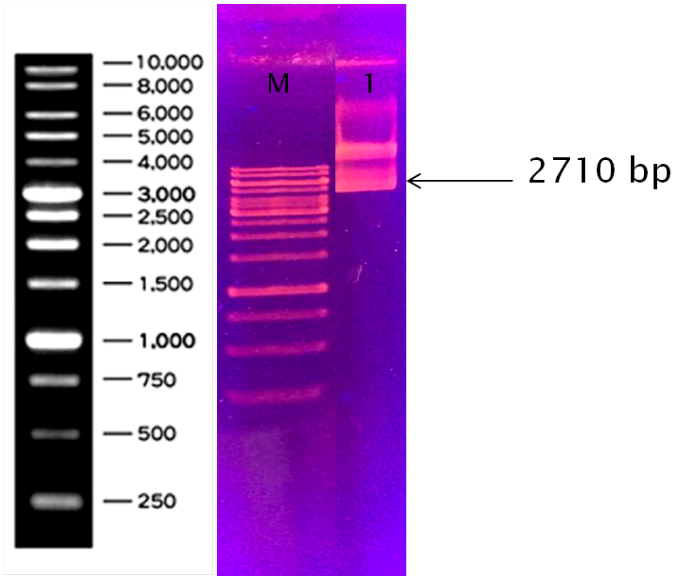

Figure 1. Purification of PUC57 plasmid

Note: Column M indicates DNA size (1 kb DNA ladder); Column 1 extracted plasmid PUC57.

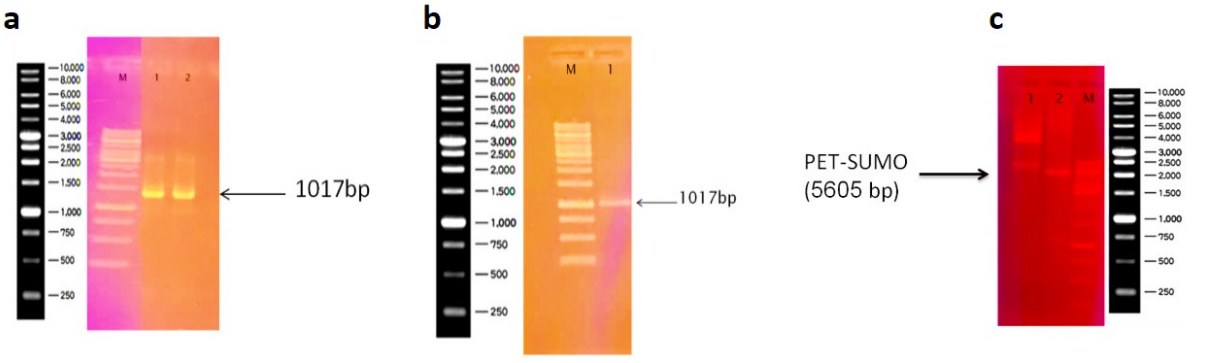

Figure 2. Amplification of the Sal k 1 gene by PCR and Pfu enzyme, enzymatic digestion of the pET-Sumo plasmid and the PCR product

A) Amplification of the *Sal k 1* gene using PCR and Pfu enzyme, B) Enzymatic digestion of the pET-sumo plasmid, C) The PCR product

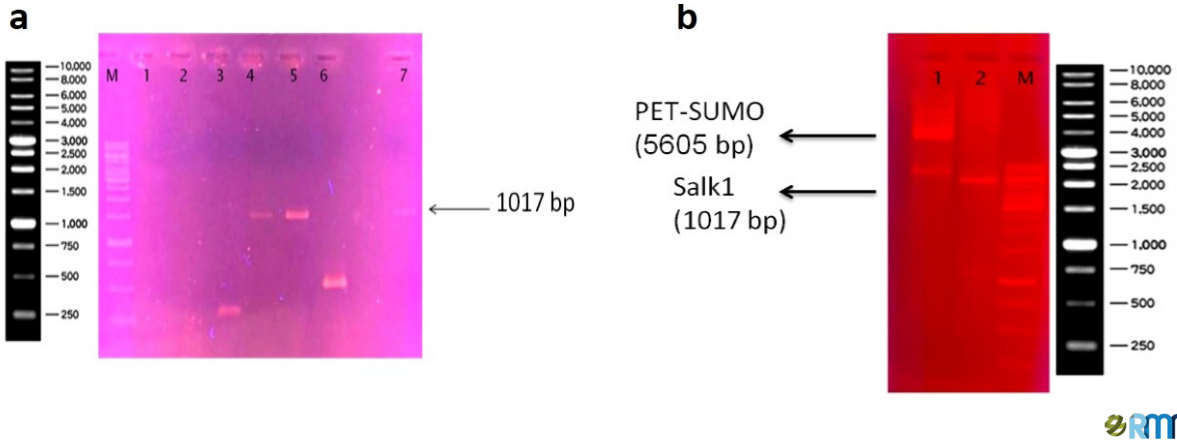

Figure 3. Screening of clones containing recombinant DNA (a), and confirmatory digestion of the extracted plasmid with XhoI and SspI enzymes (b)

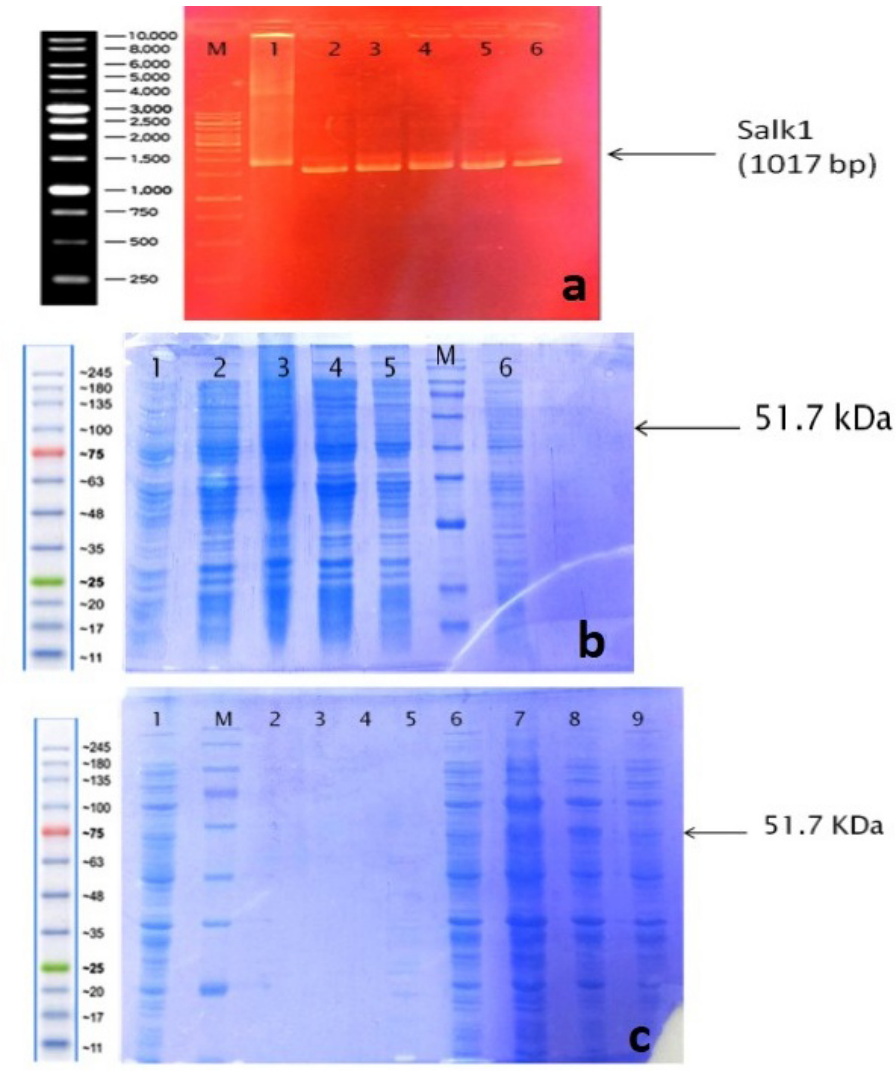

Figure 4. Transfer of the recombinant plasmid to susceptible BL21 and Rosetta cells (a) and examination of its expression with SDS-PAGE (b and c, respectively)

Purification of the recombinant protein using a combined Ni-NTA chromatography column and optimization

Purification of the recombinant protein was performed using a Ni-NTA combined molecular chromatography column and both denatured and native recombinant methods according to the Qiagen catalog. The amino-terminal His-tag was added to the amino-terminal end of the protein following cloning of the gene in the XhoI/SspI enzyme site in the pET-Sumo vector (Figure 5).

Western blotting results

Confirmation of the recombinant protein product of the *Sal k 1* gene with a molecular weight of 51.7 kDa was performed using the Western blotting method. In this method, the anti-His-tag antibody was used. The result of Western blotting of the purified recombinant protein on nitrocellulose paper is shown in Figure 6.

Indirect ELISA results

As shown in Figure 7, the performance of recombinant Sal k 1 protein was assessed using indirect ELISA.

Discussion

Allergy is an immune disorder that is caused by an over-reaction of the immune system to relatively normal and harmless antigens derived from the environment or diet, as well as substances identified as allergens [15]. Allergy

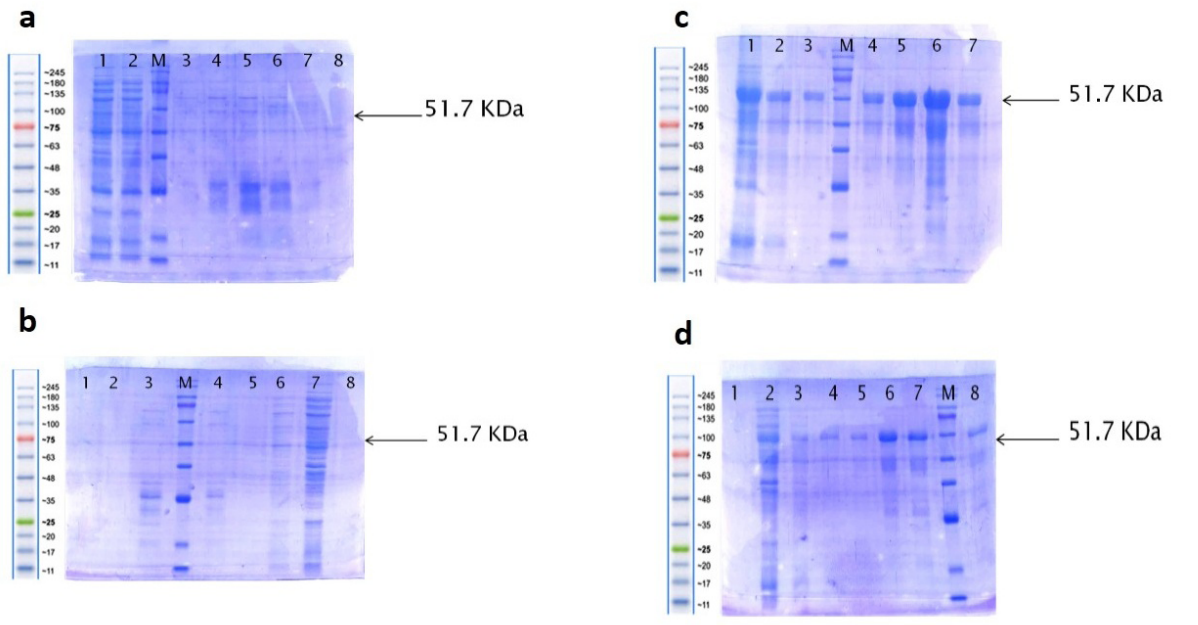

Figure 5. Purification of the recombinant protein using a combined Ni-NTA chromatography column

a) Purification of the recombinant Sal k 1 protein in Rosetta, a cell protein extract (native), b) Purification of the recombinant Sal k 1 protein in BL21 cell protein extract (native), c) Purification of the recombinant Sal k 1 protein in Rosetta cell protein extract (denatured), d) Purification of the recombinant Sal k 1 protein in BL21 cell protein extract (denatured)

is characterized as a type I hypersensitivity mediated by IgE, resulting from degranulation of mast cells and basophils [16]. Most allergens known in nature induce a Th2 response. Th2-specific cytokines induce allergen-specific IgE antibodies in sensitized individuals. Allergens bind to IgE and attach to the FCεRI receptor on mast cells and basophils, inducing the release of excessive allergenic mediators, which in turn lead to allergic signs and symptoms [17]. The best way to prevent allergies is to avoid exposure to allergens or to use antihistamines and steroids. Also, immunotherapy for desensitization to allergens is one of the effective and beneficial treatment

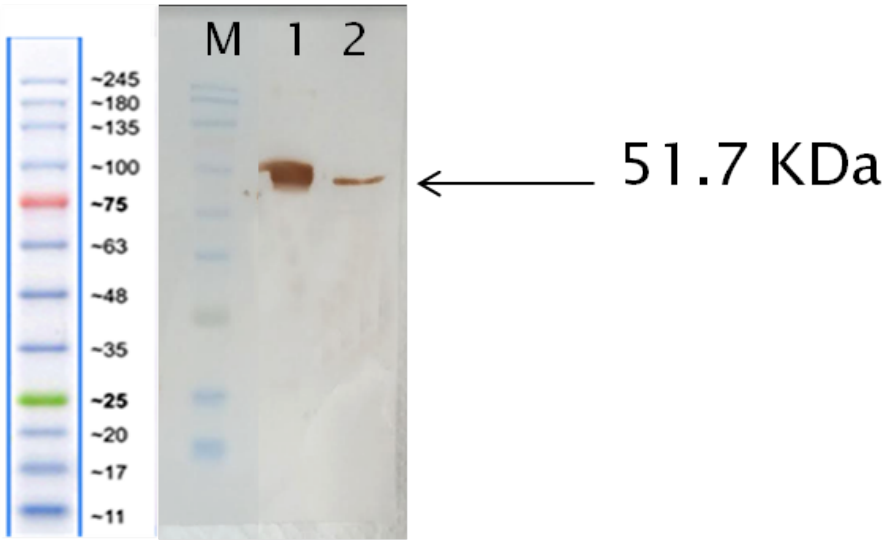

Figure 6. Confirmation of the recombinant proteins by Western blotting using an anti-His-tag antibody

Note: Column M: Protein size indicator; Column 1: Recombinant protein Sal k 1 expressed in native BL21 cells (E3); Column 2: Recombinant protein Sal k 1 expressed in denatured BL21 cells (MES).

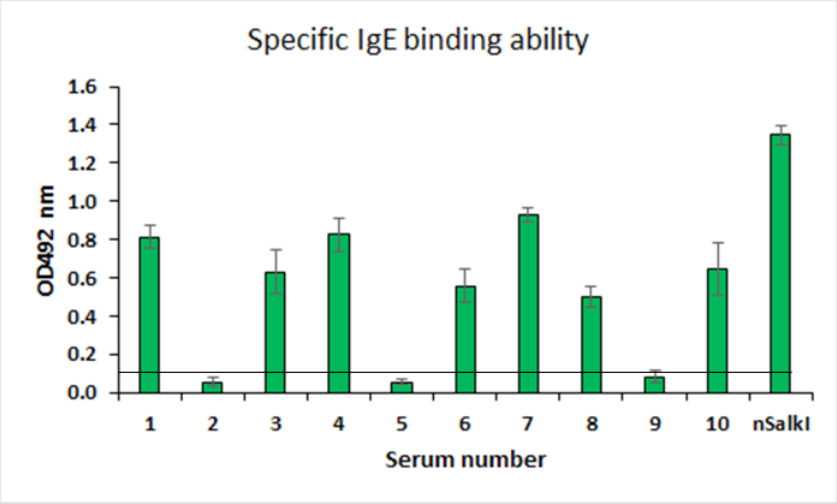

Figure 7. Evaluation of the performance of recombinant Sal k 1 protein by indirect ELISA

Note: OD<0.1 (threshold line) is considered a negative reaction. Samples 2, 5, and 9 are from individuals without sensitivity to Sal k 1 protein. Each sample was assessed three times.

methods available both now and in the future [18]. Recognizing the types of allergens in this disease and finding appropriate and effective ways to identify and treat them as harmlessly as possible is among the most important areas of research for scientists in this field [16].

The role of Amaranthaceae pollen as a source of allergens is important in desert countries with hot and dry climates, in Iran, as well as in the southern regions of Europe, which have been experiencing increased desertification in recent years. Many studies have been conducted on pollen allergens from other members of the *Salsola* sp. family, including *S. oppositifolia* and *S. vermiculata*. However, *S. kali* is considered the main allergenic pollen from *Salsola* sp. To date, 5 allergens from *S. kali* have been reported: Sal k 2 homologous protein kinase (accession number: Q8L5K9), Sal k 3 methionine synthetase, Sal k 4 profilin, Sal k 5 Ole e 1-like protein, and Sal k 1, which is one of the main allergens and also serves as a specific marker for *S. kali* [12, 13]. Ghasemi et al. were the first to clone and express the rSal k 1 protein in *Lactococcus lactis*. Ghasemi et al. used *L. lactis* as a host because it secretes the protein [19].

Accordingly, recombinant LAB was used as a mucosal safe drug carrier in allergy immunotherapy [19]. Charng et al. also showed that recombinant Sal k 1 allergen in LABs is able to reduce allergen and airway inflammatory responses. Among LABs, *L. lactis* has received much attention as a recombinant carrier of oral vaccines for allergy desensitization. The function of the recombinant protein is a determining factor in mucosal stimulation of the immune system. The expression level of the protein obtained with recombinant *L. lactis* has been shown to be much higher than that of other lactobacilli. It is worth noting that the Western blot results confirmed the immunoreactivity of the rSal k 1 protein and indicated that protein production could be detected by IgE antibodies present in the serum of patients sensitized to Sal k 1[20].

A study also tested bromelain (a protein containing carbohydrate interactions) to determine whether IgE binding to rSal k 1 and nSal k 1 could be attributed to glycan moieties not present on rSal k 1. Only 66% of patients showed direct binding of IgE to the glycosidic moieties in bromelain, and only one patient (1.1% of the population) exhibited an association between IgE reactivity and glycan moieties. These investigators suggested that the glycan moiety does not participate in the reaction with IgE. It is worth noting that previous studies on glycoallergens have shown that the glycan moiety present on nSal k 1 can lead to false binding of IgE in patients. Therefore, the use of rSal k 1 in diagnostic protocols could alleviate these problems due to the absence of glycosylation in the recombinant allergen. This study demonstrated the importance of cloning and producing recombinant Salk1 [21]. The aim of the present study was to produce and express recombinant Sal k 1 protein in *E. coli* expression host as a means to diagnose allergies and examine the intensity of IgE binding to *S. Kali* pollen. Among the 4 major allergens known from *S. kali* pollen (Sal k 1, Sal k 2, Sal k 3, and Sal k 4), Sal k 1, which exhibits low IgE binding, was selected in this study. On the other hand, Sal k 1 is the most abundant protein in *S. kali* pollen extract. Also, the results of other researchers' studies have shown that approximately 99%

of patients sensitive to *S. kali* pollen experience a severe reaction to Sal k 1 [10, 22, 23].

Conclusion

In the present study, the *Sal k 1* gene was expressed in the *E. coli* expression strain (BL21-DE3) after induction with IPTG, resulting in a band of 51.7 kDa on the SDS-PAGE gel. Protein purification was performed using a Ni-NTA combined molecular chromatography column under native conditions. In the test to examine the expression at different hours after induction with IPTG, very good expression was seen using SDS-PAGE at 16 hours. The amount of recombinant protein in denatured conditions was more appropriate than in native conditions. Finally, it is hoped that the aforementioned protein can be produced recombinantly on a large scale so that it may be used to treat allergies in individuals susceptible to this disease.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors contribution's

Data analysis and writing the original draft: Nastaran Farahani; Conceptualization, study design, and supervision, review and editing: Shohreh Zare Karizi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Yong SB, Gau SY, Guo YC, Wei JC. Allergy from perspective of environmental pollution effects: From an aspect of atopic dermatitis, immune system, and atmospheric hazards-a narrative review of current evidences. Environ Sci Pollut Res Int. 2022; 29(38):57091-101. [DOI:10.1007/s11356-022-21582-3] [PMID]

[2] Pramod SN. Immunological basis for the development of allergic diseases-prevalence, diagnosis and treatment strategies, in cell interaction-molecular and immunological basis for disease management. London: IntechOpen; 2021. [DOI:10.5772/intechopen.95804]

[3] Goodman RE, Chapman MD, Slater JE. The allergen: Sources, extracts, and molecules for diagnosis of allergic disease. J Allergy Clin Immunol Pract. 2020; 8(8):2506-14. [DOI:10.1016/j.jaip.2020.06.043] [PMID]

[4] Konstantinou GN, Konstantinou GN, Koulias C, Petalas K, Makris M. Further understanding of neuro-immune interactions in allergy: Implications in pathophysiology and role in disease progression. J Asthma Allergy. 2022; 15:1273-91. [DOI:10.2147/JAA.S282039] [PMID]

[5] Figueiredo IAD, Ferreira SRD, Fernandes JM, Silva BAD, Vasconcelos LHC, Cavalcante FA. A review of the pathophysiology and the role of ion channels on bronchial asthma. Front Pharmacol. 2023; 14:1236550. [DOI:10.3389/fphar.2023.1236550] [PMID]

[6] Shinde V, Wankhede P, Vyawahare N. Asthma: Cells involved in the pathophysiology of asthma. J Assoc Chest Phys. 2023; 11(1):10-8. [DOI:10.4103/jacp.jacp_41_22]

[7] Hammad H, Lambrecht BN. The basic immunology of asthma. Cell. 2021; 184(6):1469-85. [DOI:10.1016/j.cell.2021.02.016] [PMID]

[8] Gohal G, Moni SS, Bakkari MA, Elmobark ME. A review on asthma and allergy: Current understanding on molecular perspectives. J Clin Med. 2024; 13(19):5775. [DOI:10.3390/jcm13195775] [PMID]

[9] Ahasan F. Dust mite allergy and the significance of different immunotherapies to treat it [doctoral dissertation]. Dhaka: Brac University; 2022. [Link]

[10] Peterkova L, Trifonova D, Gattinger P, Focke-Tejkl M, Garib V, Magbulova N, et al. The cytoskeletal protein profilin is an important allergen in saltwort (Salsola kali). Front Immunol. 2024; 15:1379833. [DOI:10.3389/fimmu.2024.1379833] [PMID]

[11] Agulló-García A, Colás Sanz C, Martínez-Lostao L, Salamanca Fernández G, Ferrer Clavería L, Cubero Saldaña JL, et al. Clinical and Molecular Profiles in Patients Allergic to Amaranthaceae. J Investig Allergol Clin Immunol. 2024; 34(2):123-5. [DOI:10.18176/jiaci.0931] [PMID]

[12] Arilla MC, Ibarrola I, Brena S, Martínez A, Colás C, Asturias JA. The Russian thistle (Salsola kali) pollen major allergen, Sal k 1, can be quantified in allergenic extracts and airborne pollen. Int Arch Allergy Immunol. 2010; 152(4):319-26. [DOI:10.1159/000288285] [PMID]

[13] Villalba M, Barderas Manchado R, Mas S, Colás S, Batanero Cremades E, Rodríguez García R. Amaranthaceae pollens: Review of an emerging allergy in the Mediterranean area. J Investig Allergol Clin Immunol. 2014; 24(6):288-97. [Link]

[14] Gadermaier G, Hauser M, Ferreira F. Allergens of weed pollen: an overview on recombinant and natural molecules. Methods. 2014; 66(1):55-66. [DOI:10.1016/j.ymeth.2013.06.014] [PMID]

[15] Dotsenko SY, Sychov RO, Tokarenko II, Shevchenko MV, Chornaya IV, Kulynych OV. Clinical immunology and allergology. Zaporizhia: Zaporozhye State Medical University; 2016. [Link]

[16] He SH, Zhang HY, Zeng XN, Chen D, Yang PC. Mast cells and basophils are essential for allergies: Mechanisms of allergic inflammation and a proposed procedure for diagnosis. Acta Pharmacol Sin. 2013; 34(10):1270-83. [DOI:10.1038/aps.2013.88] [PMID]

[17] Maggi E. The TH1/TH2 paradigm in allergy. Immunotechnology. 1998; 3(4):233-44. [DOI:10.1016/S1380-2933(97)10005-7] [PMID]

[18] Mener DJ, Lin SY. Improvement and prevention of asthma with concomitant treatment of allergic rhinitis and allergen-specific therapy. Int Forum Allergy Rhinol. 2015; 5(Suppl 1):S45-50. [DOI:10.1002/alr.21569] [PMID]

[19] Ghasemi Z, Sankian M, Moghadam M, Sedghy F, Jalali A, Jabbariazad F, Varasteh AR. Production and study of immunological properties of transformed Lactococcus lactis capable of expressing Sal K1, the major allergen of Salsola kali pollen, as live vaccines for allergy treatment. Iran J Allergy Asthma Immunol. 2018; 17:125. [Link]

[20] Charng YC, Lin CC, Hsu CH. Inhibition of allergen-induced airway inflammation and hyperreactivity by recombinant lactic-acid bacteria. Vaccine. 2006; 24(33-34):5931-6. [DOI:10.1016/j.vaccine.2005.07.107] [PMID]

[21] Mas S, Boissy P, Monsalve RI, Cuesta-Herranz J, Díaz-Perales A, Fernández J, et al. A recombinant Sal k 1 isoform as an alternative to the polymorphic allergen from Salsola kali pollen for allergy diagnosis. Int Arch Allergy Immunol. 2015; 167(2):83-93. [DOI:10.1159/000434680] [PMID]

[22] Shokouhi Shoormasti R, Sabetkish N, Kazemnejad A, Vahabi N, Fazlollahi MR, Pourpak Z, et al. A meta-analysis of sensitization to the most common aeroallergens in a Middle Eastern region: An overview of the main molecular allergens. Aerobiologia. 2019; 35(3):383-409. [DOI:10.1007/s10453-019-09586-y]

[23] Tabesh S, Fanuel S, Fazlollahi MR, Yekaninejad MS, Kardar GA, Razavi SA. Design and evaluation of a hypoallergenic peptide-based vaccine for Salsola kali allergy. Int Immunopharmacol. 2019; 66:62-68. [DOI:10.1016/j.intimp.2018.10.037] [PMID]