Int J Clin Exp Med 2016;9(6):10829-10837
www.ijcem.com /ISSN:1940-5901/IJCEM0020914

Original Article

Characterization of a novel allergen Der f 25, homologous to triose-phosphate isomerase, from *Dermatophagoides farinae*

Jianli Lin[1*], Qian Wan[1*], Anjian Gao[1*], Congli Jiang[1], Yulin Liu[2], Zhigang Liu[1,2], Pingchang Yang[1], Xiaoyu Liu[1]

[1]Institute of Allergy & Immunology, Shenzhen University, Shenzhen, China; [2]The Affiliated Luohu Hospital of Shenzhen University, Shenzhen Luohu Hospital Group, Shenzhen 518000, China. [*]Equal contributors.

Received October 17, 2015; Accepted March 29, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Dermatophagoides farinae, as one species of house dust mites, was known to be rich in IgE-reactive components that contribute to allergy in China and worldwide. This study aims to characterize a novel allergen, the D. Farinae-derived Der f 25. In this study, total RNA was extracted from Dermatophagoides farinae and the full-length of Der f 25 gene was amplified by RT-PCR with the primers designed according to the sequence of Der f 25. The target gene was cloned into pUC57 vector following sequencing identification, sub-cloned into pET32a(+), and transformed into E. coli BL21 (DE3) for expression. The recombinant protein was purified by Ni-NTA affinity chromatography and analyzed by SDS-PAGE. Its allergenicity was identified by skin prick tests, Western blotting and ELISA. The sequencing indicated the cloned Der f 25 contains 744 bp encoding 247 amino acids which displaying strong homology with triose-phosphate isomerase Dermatophagoides farinae (Gen Bank AGC56216.1). Patients from 16 of 42 house dust mite allergy subjects (38%) showed positive reaction to Der f 25. And 4 positive sera obtained showed strong IgE binding ability to the recombinant protein Der f 25. IgE binding to the house dust mites extracts can be inhibited by recombinant Der f 25 in a dose-dependent manner. In conclusion: A new allergen (Der f 25) was identified from Dermatophagoides farinae which also has the similar immunologic activity to natural Der f 25 protein.

Keywords: Dermatophagoides farinae, Der f 25, cloning, SPT, ELISA

Introduction

Allergic diseases are increasing worldwide with unprecedented complexity and severity especially in children. These diseases include asthma, rhinitis, anaphylaxis for drug, food, and insect, eczema, and urticaria (hives) and angioedema. According to epidemiological statistics, allergic rhinitis alone currently affects between 10% and 30% of the population [1]. So allergy should be recognized as a public health problem and efforts should be made towards its prevention and optimal treatment.

House dust mites (HDMs) Dermatophagoides pteronyssinus and farinae are predominant sources of inhalant allergens, with more than 50% of allergic disease cases being attributed to them [2-5]. Up to now, 16 allergens from D. farinae (Der f1-3, 5-7, 10, 11, 13-18, 22, 24) have been named [6]. As a novel D. farinae allergen, the identification of Der f 25 will be helpful for HDM allergy diagnosis and therapy.

Some of the approaches for the treatment of allergic diseases are pharmacotherapy, allergen specific immunotherapy and biological agents. Allergen specific immunotherapy (AIT) is considered as an effective treatment for respiratory allergy and Hymenoptera venom allergy. Subcutaneous Immunotherapy (SCIT) represents the standard modality of treatment [1]. Conventional SIT is often used to treat patients by injecting increasing amounts of a diluted mite allergen over several months [7]. At present, the dust mites extracts used in immunotherapy of allergic patients are recognized to be easily leading to some side effects in clinical settings due to its complicated compositions [8, 9]. Based on the advantage of no exogenous pollution, high purity, easy standardization compared with the crude extract of dust mites, the

recombinant allergen vaccine was used in clinical immunotherapy. Using the genetic engineering techniques to product more major allergens *in vitro* is an important way in allergic disease diagnosis and desensitization.

In the present study, Der f 25 was cloned, expressed in E. coli, refolded by dilution methods, and purified by affinity chromatography. Furthermore, the allergenicity of recombinant protein Der f 25 was detected by skin prick tests *in vivo* and Western blotting, ELISA assay *in vitro*.

Materials and methods

Cloning and sequencing of Der f 25

House dust mite were cultured and isolated by our research group according to Sasa's method [10]. Total RNAs were obtained using RNeasy mini kit (Qiagen) after liquid nitrogen grinding and reverse transcription (RT) was performed with TaKaRa PrimeScriptTM RT-PCR Kit (Code No. DR014A, TaKaRa).

The gene sequence of Der f 25 was obtained by blast with the whole genome of Dermatophagoides farina. (GenBank Accession KM010004.1), a pair of specific primers was designed as follows: 5'-GGATCCATGGGTCGCAAATTCTTCG-3' (forward) and 5'-GAATTCTTATTGACGAGCGTTAAC-3' (reserve) with an EcoR I site and a BamH I site at their 5'ends (underlined), respectively. The sequences were synthesized by Sangon Biotech Limited Company, Shanghai, China. The RT-PCR product was used as the template for PCR in the thermal cycler with PrimeSTAR® HS DNA Polymerase (TaKaRa, DR-044A). The total reaction mixture contained RT products (2 μL), 5 × PrimeSTAR PCR buffer (10 μL), 2.5 mmol/L of dNTP mixture (4 μL), 20 μmol/L of forward primer (0.5 μL), 20 μmol/L of reserve primer (0.5 μL), 2.5 U/μL of PrimeSTAR HS DNA polymerase (0.25 μL), and dH_20 (31.5 μL). PCR conditions used here included an initial incubation for 5 minutes at 94°C, and followed by 31 cycles of 30 seconds at 94°C, 30 seconds at 51°C and 90 seconds at 72°C. After a final incubation for 5 minutes at 72°C, 5 μL of the PCR product were analyzed by agarose electrophoresis (1.2%) and visualized with Molecular Imager™ (Bio Rad, Gel Doc XR+). The PCR-amplified DNA was then cloned into pUC57 with the DNA Ligation Kit (TaKaRa Code No. D6020A). E. coli Top10 cells were transformed with the recombinant plasmid pUC57-Der f 25, positive clones were selected by blue/white screening on Luria-Bertani (LB) plates containing 100 μg/mL ampicillin. And samples were submitted to Shanghai Sangon Company for DNA sequencing.

Expression and purification of recombinant Der f 25

After sequencing, the verified pUC57-Der f 25 plasmid and the expression vector pET32a (+) (Kit Lot No. N72770, Novagen, Germany) were digested with EcoR I and BamH I to link with each other using the DNA Ligation Kit (Code No. D6023, TaKaRa). Competent E. coli Top10 cells (Code No. D9052, TaKaRa) were transformed with pET32a(+)-Der f 25 plasmids, positive clones were selected by blue/white screening, confirmed by restriction enzyme analysis with EcoR I and BamH I and sequenced by Sangon Biotech Limited Company, Shanghai, China.

Purified pET32a(+)-Der f 25 plasmids were used to transforminto E. coli BL21 (DE3). The pET32a(+)-Der f 25-carrying E. coli BL21 were grown on LB plates containing 50 μg/mL ampicillin at 37°C overnight. A single colony was inoculated into 20 mL LB fluid containing 20 μL ampicillin at 37°C, 150 rpm/min in constant temperature oscillation incubator overnight. One mL bacteria liquid was added into a glass tube containing 1 L LB and then cultured at 37°C with shaking 200 rpm/min until the OD600 reaches 0.6. 1 mM Isopropyl-β-D-thiogalactopyranoside was added to induce the expression at 4°C, 200 rpm/min for 4 h. The samples of bacteria liquid was analyzed by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE, 12%).

Cell pellets of inclusion bodies were resuspended in 30 mL 1 × PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 8.0) and subjected to sonication for 10 min with 2 s intervals. The cell lysate was then centrifuged at 12,000 rpm for 25 min at 4°C and the inclusion body pellet was dissociated in 1 × binding buffer containing 6 M urea. Upon filtration, the recombinant proteins were purified using Ni-nitrilotriacetic acid-agarose (GE, USA) according to the manufacturer's instructions. Proteins were dialyzed against refolding buffer (0.1 M Tris, pH 8.0, 0.4 M L-Arginine, 0.5 mM

oxidized glutathione, 5 mM reduced glutathione). After dialysis, the refolded protein was centrifuged and the supernatant was purified by Ni-NTA column as mentioned above. The elution peak was collected and loaded to 3 kDa 500 μL Millipore Centrifugal Filters to concentrate. The concentrated protein was purified by molecular sieve chromatography and assessed by SDS-PAGE for the purity.

Skin prick tests and serum samples

SPTs with recombinant protein Der f 25 were performed by using the single-prick technique according to the method of Dreborg [11]. Negative (sodium chloride) and positive (histamine, 5 mg/mL) controls were used for the comparison. Skin response was observed after 20 min and defined as positive when the presence of a wheal over the skin and showing spots around it. 42 patients who claimed allergy to dust mites and SPTs with DME are positive participated in the SPTs. All the manipulations were approved by the patients as well as the local medical and ethics authorities.

Sera were obtained from 4 Der f 25 allergic patients who were diagnosed with allergic rhinitis or allergic asthma in clinic in the Allergic response department of the Second Affiliated Hospital of Guangzhou Medical University, Guangdong province, China. The sera of 2 healthy people were used as negative controls in the following assays. All the manipulations were approved by the patients as well as the local medical and ethics authorities.

Western blotting

The purified recombinant fusion protein was first applied to SDS-PAGE (150 μg per-well) and then electrophoretically transferred to nitrocellulose membrane. The membrane was then cut into small strips of about 4 mm wide and blocked in blocking buffer (Tris-buffered saline solution containing 5% BSA and 0.1% Tween 20, TBST, pH 7.6). After blocking for 1 h, the membranes were incubated with sera from 4 positive patients in the SPTs (1:5 diluted in TBST) as well as control sera from 2 healthy people. After being washed 3 times, the HRP-labeled mouse anti-human IgE antibody (southern biotech; 1:2,000 diluted in TBST) were sequentially added. Lastly, the bands were visualized by using ECL (Bio Rad) as substrate.

ELISA and ELISA inhibition assay

Sera IgE antibodies specific for purified Der f 25 was measured by ELISA. Briefly, 100 ng Der f 25 diluted in 100 μL coating buffer (15 Mm Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6) was added into a 96-well microtiter plate and incubated at 4°C overnight. After being blocked with 200 μL 3% BSA in PBS at 37°C for 1 h and washed three times, 100 μL sera from patients 1-4 and 2 healthy controls (1:30 diluted with 3% BSA-PBST) were added and incubated for 1h at 37°C. The bound IgE was then reacted with 100 μL HRP-labeled mouse anti-human IgE antibody (Southern biotech; 1:2,000 diluted in PBST). TMB was used as the substrate and the reaction was stopped by 50 μL 2 M H_2SO_4. The OD 450 of each well was measured using an ELISA microplate reader (Bio-Rad). The cutoff value for positive ELISA results was set as the mean value plus 4 standard deviations (SD) of the sera from the 2 healthy controls. For the inhibition assay, pooled sera from patients were pre-incubated with inhibitors of Der f 25 or crude D. farinae extract at different concentrations (1, 10, 100 and 1,000 μg/mL) for 2 h at 37°C and then reacted with the coated crude D. farinae extract as antigen. Data from 3 measurements were accumulated. The inhibition rates were calculated according to the following formula: inhibition (%) = $(OD_0-OD_{inhibitor})/(OD_0-OD_{BSA})$, where OD_0 is the optical density of antigen without any inhibitor, $OD_{inhibitor}$ is the optical density after adding an inhibitor and OD_{BSA} is the optical density with only BSA in the plate [12].

Data statistics

All data were expressed as means ± SD and processed with Graphpad software, and the T-test was used for the mean differences between two groups. *, $P<0.05$. **, $P<0.01$.

Results

Obtaining of full-length Der f 25 and construction of expression plasmids pET32a(+)-Der f 25

Total RNA was extracted from dust mites, and Der f 25 cDNA fragments were amplified by PCR. A band size indicating the expected 744 base pairs was spotted following gel electrophoresis (**Figure 1A**). After the PCR-amplified

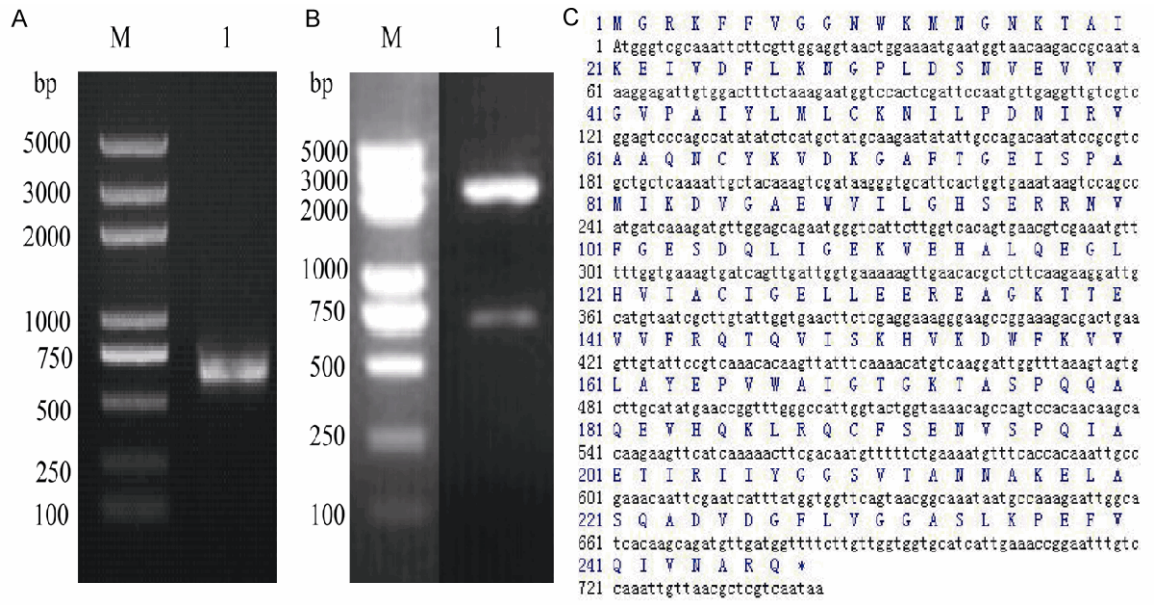

Figure 1. Cloning and sequencing of cDNA encoding Der f 25. A. Amplification of the cDNA of the gene encoding Der f 25 protein by RT-PCR. Lane M, DNA Marker DL2, 000; Lane 1, PCR product (744 bp). B. Restriction enzymes digestion analysis of Recombinant plasmid pET-32a(+)-Der f 25. C. cDNA sequence encoding Der f 25 and the deduced amino acid sequence. *: Stop codon. Lane M: DL2000 DNA Marker; Lane 1: Plasmid pET-32a(+)-Der f 25 was digested by EcoR I and BamH I.

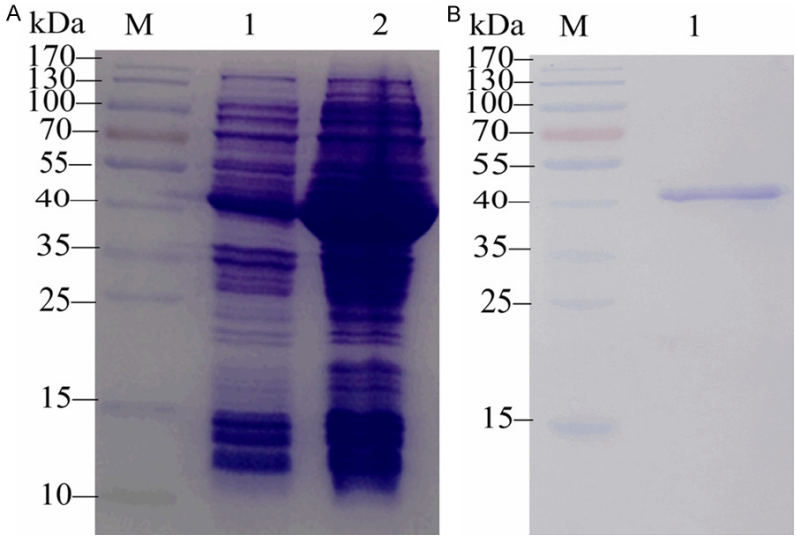

Figure 2. The expression and purification of recombinant protein Der f 25. A. SDS-PAGE analysis of the protein expressed from the pET-32a(+)-Der f 25 recombinant plasmid in E. coli BL21 cells. Lane M: Fermanters Protein Marker (0671); Lane 1: Ecoli Origami pET-32a(+)-Der f 25 before IPTG induction; Lane 2: Ecoli Origami pET-32a(+)-Der f 25 after induction for 4 h; B. SDS-PAGE analysis of the final purified refolded recombinant protein from E. coli BL21 cells. Lane M: Fermanters Protein Marker (0671). Lane 1: Refolded recombinant protein Der f 25 after purification.

DNA was recovered, the product was cloned into vector pUC-57T and transformed into E. coli competent cells (E. coli Top10). The product was confirmed by Sangon Biotech Limited Company, Shanghai, China. Following sequencing, a complete ORF was found, stretching 744 bp from the start codon ATG to the stop codon TAA and encoding 247 amino acids (**Figure 1C**). And then the positive recombinant expression plasmids were extracted and verified by agarose gel electrophoresis (**Figure 1B**). The full length and sequence of nucleotide acid of the inserted gene in extracted recombinant plasmid was completely identical to expectations.

Expression and purification of recombinant protein Der f 25

The verified recombinant plasmids pET-32a(+)-Der f 25 was transformed into E. coli BL21, and

Table 1. Results of skin prick tests

Subject	Gender/Age	Diagnosis	Net wheal size (mm), Level			
			DME	Histamine	PS	r-Der f 25
1	Male/11	BA+AR	9, 3+	6	0	2, +
2	Female/50	AR	2.25, +	5	0	0
3	Female/25	AR	6.75, 3+	3.75	0	0
4	Male/16	AR	2, +	6.5	0	0
5	Male/43	BA+AR	6, 2+	6	0	2.75, +
6	Male/26	AR	5, 2+	6	0	0
7	Male/32	AR	1.75, +	6	0	0
8	Male/12	AR	4.5, 2+	4.75	0	3.75, 2+
9	Male/46	BA+AR	4.5, 2+	8.5	0	0
10	Female/17	AR	4.5, 3+	4.5	0	0
11	Male/45	BA	3, +	7	0	2.5, +
12	Male/19	BA	0.5, +	1	0	0
13	Male/38	BA+AR	6, 2+	7.5	0	0
14	Male/8	BA+AR+FA	10, 3+	7	0	1.25, +
15	Female/19	BA+AR+U+DA	1, +	6.5	0	0
16	Female/47	BA+AR+FA+DA	4.5, 2+	8	0	1.5, +
17	Female/37	AR	2.5, 2+	5	0	1.75, +
18	Female/33	AR	1.5, +	5.75	0	0
19	Male/24	BA+AR	2, +	5	0	0
20	Male/33	BA	10, 3+	6.5	0	2.5, +
21	Male/36	AR	7.5, 3+	5	0	2, +
22	Female/43	BA	3.5, 2+	5	0	0
23	Female/51	BA+AR	2.5, 2+	4.5	0	0
24	Female/18	BA+AR+FA+DA	3.5, 3+	2	0	0
25	Female/42	BA+AR+FA+DA	3.5, 2+	5	0	1.5, +
26	Female/47	BA+AR+DA	3, 2+	6	0	0
27	Male/48	BA+AR	5.5, 3+	4.5	0	0
28	Male/15	BA+AR	6, 2+	7.75	0	0
29	Female/39	AR	12.5, 3+	8.5	0	2, +
30	Male/63	BA+DA	8.4, 3+	8	0	0
31	Male/15	BA+AR+FA	6.5, 3+	6	0	0
32	Male/12	BA+AR	11, 3+	6	0	0
33	Female/18	BA+AR	5.5, 3+	4.5	0	0
34	Female/25	AR	6.5, 3+	6.5	0	1.5, +
35	Female/40	BA+AR	6.5, 3+	5	0	0
36	Male/75	BA+AR+FA	2.5, +	6.5	0	2.5, +
37	Male/11	BA+AR+FA	6.5, 3+	5.5	0	0
38	Female/26	BA	1, +	5.5	0	1, +
39	Male/15	BA+AR+U	7, 2+	8	0	2, +
40	Female/14	BA+AR	6.5, 3+	6.5	0	0
41	Male/8	AR+FA	1, +	4.5	0	0
42	Female/43	BA	2, +	4.5	0	0

Patients with mite allergy; Gender: M: Male; F: Female. Diagnose: BA: bronchial asthma; AR: allergic rhinitis; FA: Food allergy; DA: Drug allergy; U: urticaria. Net wheal: 4+ for net wheal size larger than positive control; 3+ for reaction intensity is similar to positive control, and with the positive reaction; 2+ for results are slightly larger than positive control, but stronger than negative control; 1+ significantly weaker than positive control, but slightly stronger than the negative control; No allergic reaction to the negative (-).

expression was induced by IPTG treatment (**Figure 2A**). After NI-NTA chromatography and refolding, approximately 4.7 mg of the recombinant product was obtained. A protein band matching the postulated molecular weight was detected by SDS-PAGE (**Figure 2B**).

Skin prick testing

The allergenicity of rDer f 25 was evaluated by SPT. Sixteen of 42 (38%) dust mite allergic patients showed positive results to this new allergen (**Table 1**).

Immunology assay

Seras from 4 Der f 25 allergic patients were collected and 2 healthy individuals were also obtained as negative controls. Western blot analysis of patient sera was used to identify patterns of IgE reactivity to recombinant protein Der f 25. As the results shown, all patient sera demonstrated reactivity toward bands around 50 kDa, in contrast, all sera samples from healthy individuals exhibited no reactivity to recombinant protein Der f 25 (**Figure 3A**). The reactivity of patient sera to rDer f 25 was also examined by ELISA assay. The positive reactions are three times than the negative (**Figure 3B**). For ELISA inhibition assay, different concentrations of Der f 25 or DME were incubated with the serum. They inhibited the patients' serum IgE binding to the coated DME in a dose-dependent manner (**Figure 3C**).

Homology with other protein sequences

The calculated molecular weight of the mature protein

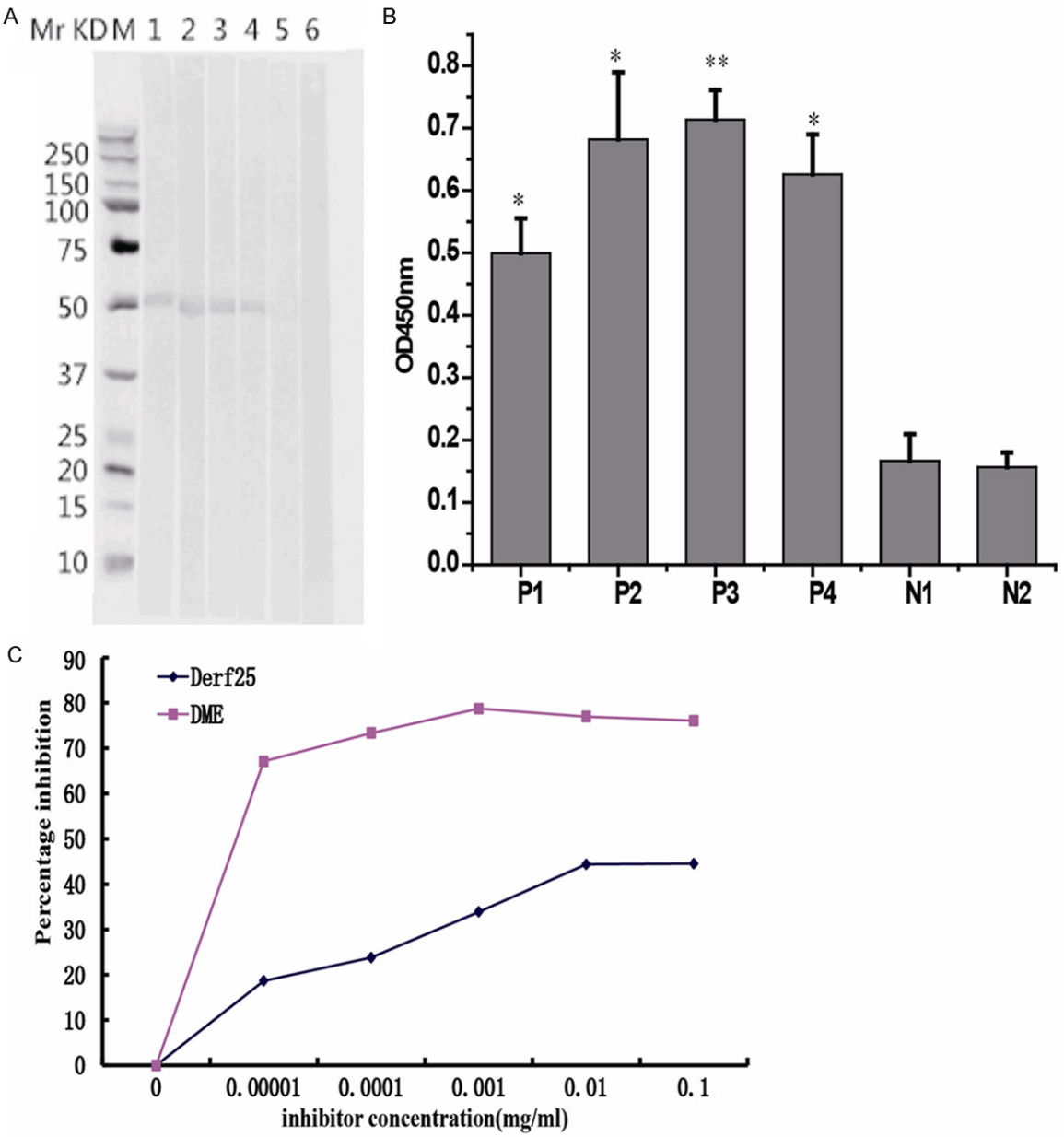

Figure 3. Immunology assay of r-Der f 25. A. Western blot assay with sera from 4 patients and 2 healthy controls. Lane M: Precision Plus Protein WesternC (Bio Rad, 161-0376); Lanes 1-4: patients who were sera positive by r-Der f 25; Lanes 5-6: sera from healthy individuals. B. Evaluation of specific IgE reactivity to r-Der f 25 by ELISA. P1-4: the sera from 4 mite-allergic patients; N1-2: 2 healthy control subjects. "*" shows statistical difference ($P<0.05$); "**" shows statistical difference ($P<0.01$). C. Purified allergens inhibited the patients' serum IgE binding to the coated DME in a dose-dependent manner. Allergen concentrations ranged from 0 to 0.1 mg/mL. DME: dust mite extracts.

is 27.1 kDa, with a predicted isoelectric point (PI) of 6.24. BLASTp protein sequence alignment (NCBI) identified similar sequences in several other species: It showed 100% homology with triose-phosphate isomerase (TPI) of *Dermatophagoides farinae* (Gen Bank AGC56-216.1), 74% identity with *Ixodes scapularis* (Gen Bank XP002411305.1), 85% identity with *Apis florea* and *Apis mellifera*, 84% identity with *Wasmannia auropunctata* (**Figure 4**).

Discussion

The group 25 allergen of Dermatophagoides farinea has been reported to be a triosephosphate isomerase (TPI). TPI is an enzyme (EC

```
Api-f   -MGRKFFVGGNWKMNGTKSEINDIVSFLKKGPLDSNVEVVVGVPSIYLTYAKNILPNNIS 59
Api-m   -MGRKFFVGGNWKMNGTKSEINDIVGFLKKGPLDSNVEVVVGVPSIYLTYAKNILPNNIS 59
Was-a   -MGRKFFVGGNWKMNGTKSEISDIVTFLKTGPLDPNVEVVVGVPSIYLTYVTSIVPSNVN 59
Der-f   -MGRKFFVGGNWKMNGNKTAIKEIVDFLKNGPLDSNVEVVVGVPAIYLMLCKNILPDNIR 59
Ixo-s   MSGRKFCVGGNWKMNGNKSSIKEICDMLKTAKLDPNTEVVLGCPAPYLDYVRRILPAAIA 60
          **** *********.*: *.:*  :**.. **.*.***:* *: **     *:*  :

Api-f   VAGQNTYKVAKGAFTGEISPAMLLDNGIPWVILGHSERRNIFGENDELIAEKVAHALESG 119
Api-m   IAGQNTYKVAKGAFTGEISPAMLLDNGIPWVILGHSERRNIFGENDELIAEKVAHALESG 119
Was-a   VSAQNTYKVAKGAFTGEISPAMLLDNGIPWVILGHSERRNIFGETDELIAEKIAHALEAG 119
Der-f   VAAQNCYKVDKGAFTGEISPAMIKDVGAEWVILGHSERRNVFGESDQLIGEKVEHALQEG 119
Ixo-s   VSAQNCYKVEKGAFTGEISPAMIKDCGATWVILGHSERRNVFKESDELIGDKVHHALESG 120
        ::.** *** ************: * *  ***********:* *.*:**.:*: ***: *

Api-f   LKVIACIGEKLEEREAGKTEEVVFRQTKAIANKIKSWDNVVVAYEPVWAIGTGKTATPQQ 179
Api-m   LKVIACIGEKLEEREAGKTDEVVFRQTKAIANKINSWDNVVVAYEPVWAIGTGKTATPQQ 179
Was-a   LKVIACIGEKLEEREAGKTEEVVYRQTKAIADKIKSWDNVVLAYEPVWAIGTGKTATPQQ 179
Der-f   LHVIACIGELLEEREAGKTTEVVFRQTQVISKHVKDWFKVVLAYEPVWAIGTGKTASPQQ 179
Ixo-s   LNVIACIGELLEEREAGKTEEVVYRQTAAIAAKVTDWNRVVLAYEPVWAIGTGKTASPEQ 180
        *:******* ********* ***:*** .*: ::..* .**:***************:*:*

Api-f   AQEVHEKLRNWFSKNVNQTVAETIRIIYGGSVTAGNAKDLAKEKDIDGFLVGGASLKPDF 239
Api-m   AQEVHEKLRNWFSKNVNQTVAETVRIIYGGSVTAGNAKDLAKEKDIDGFLVGGASLKPDF 239
Was-a   AQEVHEKLREWLSKNIKPDVAQTLRIIYGGSVTAANAKDLAKEKDIDGFLVGGASLKPDF 239
Der-f   AQEVHQKLRQCFSENVSPQIAETIRIIYGGSVTANNAKELASQADVDGFLVGGASLKPEF 239
Ixo-s   A-EVHAQLRQWLSKNVSPDVAKKVRIQYGGSVTAANCQELAKKPDVDGFLVGGASLKPEF 239
        * *** :**: :*:*:.  :*:.:** ******* *.::**.: *:**************:*

Api-f   VQIVNAKQ 247
Api-m   VQIVNAKQ 247
Was-a   VQIVNARC 247
Der-f   VQIVNARQ 247
Ixo-s   VEIINARQ 247
        *:*:**:
```

Figure 4. Characterization and homology analysis of Der f 25: Alignment between Der f 25 and its homologous amino acid sequence by Bioedit. Notes: 'Asterisks' means that the residues or nucleotides in that column are identical in all sequences in the alignment; 'Colon' indicates conserved substitutions; 'Dot' indicates semi-conserved substitutions.

5.3.1.1) that catalyzes the reversible interconversion of the triose phosphate isomers dihydroxyacetone phosphate and D-glyceraldehyde 3-p [13] hosphate [13]. TPI exists almost in all organisms, plays an important role in glycolysis and is essential for efficient energy production. Furthermore, some TPIs have been affirmed as an allergen in fish, midges, crustaceans, and various plants [14-20].

In the present study, we found that, after cloning, expression, and purification of full-length recombinant Der f 25. Der f 25 is encoded by a 744 bp open reading frame, producing a predicted amino acid sequence of 247 amino acids, with a molecular weight of 27 kDa, showing the ability of combing with the specific IgE increased by 3 folds, and 38% positive reaction by skin prick to dust mite allergic patients. This indicates Der f 25 is an important allergen for mite anaphylactic reactions. The results also show homology between the predicted sequence of Der f 25 and TPI, the results showed 100% homology with triose-phosphate isomerase Dermatophagoides farinae (Gen Bank AGC56216.1).

In conclusion, we demonstrated that the cloning, expression, and characterization of recombinant Der f 25 have enabled an initial evaluation of its potency as an allergen in mite-allergic individuals. This work lays the foundation for further study on the mechanisms of the Der f 25 allergic reaction and the diagnosis and therapy of allergic diseases, especially for patients without response to HDM major allergens. It may provide more insights to understand the diversity, characterization and allergy mechanism of D. farinae allergens.

However, the r-Der f 25 is expressed in the form of insoluble polypeptide aggregates in E. coli, therefore, the large-scale production of r-Der f 25 that is biologically functional *in vitro* be-

comes a central problem that need further to be solved.

Acknowledgements

This study was supported by grants from the Natural Science Foundation of China (No. 91-442118, 31328014 and 31400786), Guangdong Scientific Technology Social Development Project (No. 2013B3180002), Guangdong Foreign Scientific Technology Cooperative Project (No. 2013B051000088), Shenzhen Scientific Technology Project International Cooperative Project (GJHZ20130408174112021), Shenzhen Scientific Technology Basic Research Projects (No. JCYJ20140828163633991, JCYJ20130329110735981, JCYJ201505250929-41036, JCYJ20140828163633992).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Xiaoyu Liu and Pingchang Yang, Institute of Allergy & Immunology, Shenzhen University, Room 722 of Medical School Bldgn, 3688 Nanhai Blvd, Shenzhen 518060, China. Tel: 8675526681907; Fax: 8675526681906; E-mail: lxy0901@163.com (XYL); pcy2356@szu.edu.cn (PCY)

References

[1] Pawankar R, Canonica GW, Holgate ST, Lockey RF, Organization WA. World Allergy Organization (WAO) white book on allergy. Milwaukee (WI): World Allergy Organization; 2013.

[2] Voorhorst R, Spieksma FTM, Vareka mp H, Leupen MJ, Lyklema AW. The house-dust mite (Dermatophagoides pteronyssinus) and the allergens it produces. Identity with the house-dust allergen. J Allergy 1967; 39: 325-339.

[3] Lin J, Li M, Liu Y, Jiang C, Wu Y, Wang Y, Gao A, Liu Z, Yang P, Liu X. Expression, purification and characterization of Der f 27, a new allergen from dermatophagoides farinae. Am J Transl Res 2015; 7: 1260-1270.

[4] Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM. Characterization and immunobiology of house dust mite allergens. Int Arch Allergy Immunol 2002; 129: 1-18.

[5] Chan TF, Ji KM, Yim AK, Liu XY, Zhou JW, Li RQ, Yang KY, Li J, Li M, Law PT, Wu YL, Cai ZL, Qin H, Bao Y, Leung RK, Ng PK, Zou J, Zhong XJ, Ran PX, Zhong NS, Liu ZG, Tsui SK. The draft genome, transcriptome, and microbiome of Dermatophagoides farinae reveal a broad spectrum of dust mite allergens. J Allergy Clin Immunol 2015; 135: 539-548.

[6] An S, Chen L, Long C, Liu X, Xu X, Lu X, Rong M, Liu Z, Lai R. Dermatophagoides farinae allergens diversity identification by proteomics. Mol Cell Proteomics 2013; 12: 1818-1828.

[7] Chen ZG, Li M, Chen YF, Ji JZ, Li YT, Chen W, Chen FH, Chen H. Effects of Dermatophagoides pteronyssinus allergen-specific immunotherapy on the serum interleukin-13 and pulmonary functions in asthmatic children. Chin Med J 2009; 122: 1157-1161.

[8] Mauro M, Russello M, Alesina R, Sillano V, Alessandrini A, Dama A, Passalacqua G, Senna G. Safety and pharmacoeconomics of a cluster administration of mite immunotherapy compared to the traditional one. Eur Ann Allergy Clin Immunol 2006; 38: 31-34.

[9] Lin JL, Wang YY, Xiao XJ, Wu YL, Sun BQ, Gao AJ, Liu ZG, Li J, Yang PC, Liu XY. Characterization of a new subtype of allergen in dermatophagoides farinae-Der f 28. J Thorac Dis 2015; 7: 1842-1849.

[10] Sasa M, Miyamoto J, Shinohara S, Suzuki H, Katsuhata A. Studies on mass culture and isolation of *Dermatophagoides farinae* and some other mites associated with house dust and stored food. J Exp Med 1970; 40: 367-372.

[11] Dreborg S, Foucard T. Allergy to apple, carrot and potato in children withbirch pollen allergy. Allergy 1983; 38: 167-172.

[12] Liu Z, Xia L, Wu Y, Xia Q, Chen J, Roux KH. Identification and characterization of an arginine kinase as major allergen from silkworm (Bombyx mori) larvae. Int Arch Allergy Immunol 2009; 150: 8-14.

[13] Wierenga RK, Kapetaniou EG, Venkatesan R. Triosephosphate isomerase: a highly evolved biocatalyst. Cell Mol Life Sci 2010; 67: 3961-3982.

[14] Bauermeister K, Wangorsch A, Garoffo LP, Reuter A, Conti A, Taylor SL, Lidholm J, Dewitt AM, Enrique E, Vieths S, Holzhauser T, Ballmer-Weber B, Reese G. Generation of a comprehensive panel of crustacean allergens from the North Sea Shrimp Crangon crangon. Mol Immunol 2011; 48: 1983-1992.

[15] Chen YH, Lee MF, Lan JL, Chen CS, Wang HL, Hwang GY, Wu CH. Hypersensitivity to Forcipomyia taiwana (biting midge): clinical analysis and identification of major For t 1, For t 2 and For t 3 allergens. Allergy 2005; 60: 1518-1523.

[16] Hoppe S, Steinhart H, Paschke A. Identification of a 28 kDa lychee allergen as a triose-phosphate isomerase. Food Agric Immunol 2006; 17: 9-19.

[17] Nakamura R, Satoh R, Nakajima Y, Kawasaki N, Yamaguchi T, Sawada J, Nagoya H, Teshima

R. Comparative study of GH-transgenic and non-transgenic amago salmon (Oncorhynchus masou ishikawae) allergenicity and proteomic analysis of amago salmon allergens. Regul Toxicol Pharmacol 2009; 55: 300-308.

[18] Pastor C, Cuesta-Herranz J, Cases B, Pérez-Gordo M, Figueredo E, de las Heras M, Vivanco F. Identification of major allergens in watermelon. Int Arch Allergy Immunol 2009; 149: 291-298.

[19] Sudha VT, Arora N, Gaur SN, Pasha S, Singh BP. Identification of a serine protease as a major allergen (Per a 10) of Periplaneta americana. Allergy 2008; 63: 768-776.

[20] Sander I, Flagge A, Merget R, Halder TM, Meyer HE, Baur X. Identification of wheat flour allergens by means of 2-dimensional immunoblotting. J Allergy Clin Immunol 2001; 107: 907-913.