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Characterization of Two Pollen Allergens of the London Plane Tree in Shanghai

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ABSTRACT

Platanus acerifolia, London plane tree, a significant source of airborne allergens, is widely grown in Shanghai and other cities in China. In recent decades, little has been known regarding the influence of the allergens on sensitizing the population in the Shanghai area. The aims of this study were to purify and characterize the two major allergens and to confirm the immunological activities of these pollen allergens in Shanghai.

Crude extract was purified with a HiTrap SP column and a Sephedex G75 column. Immunodetection was performed with ELISA and immunoblotting. Following gel proteolytic digestion and mass spectrometry, the tandem MS (MS/MS) peptide mass fingerprint was obtained and the MASCOT search engine was used to identify the peptide. The accession number of the interesting homologous data and all the sequence information was acquired by an internet database and the evolutionary trees were drawn with Mega 4.0 software.

Two proteins with molecular weights of 43 kDa and 18 kDa were purified from *P. acerifolia* pollen extract. The purified proteins were identified as pollen allergen Pla a 1 and Pla a 2 via mass spectrometry. The proteins have immunological activities with human IgE antibodies. According to the ELISA results, 12% (5/41) of the subjects were sensitive to Pla a 1 and 9% (4/41) were sensitive to Pla a 2.

Pla a 1 and Pla a 2 are thus important allergens for patients with an allergic reaction to *P. acerifolia* pollen in Shanghai.

Keywords: London plane tree; Mass spectrometry; Pla a 1; Pla a 2; *Platanus acerifolia*; Pollen allergen

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INTRODUCTION

Platanus acerifolia, London plane tree, is a ubiquitous street and courtyard shade tree.

Because of its resistance to dust, diseases and environmental pollution, many *P. acerifolia* specimens are planted as alee trees in Shanghai. During the *P. acerifolia* spring pollination period, pollen dispersal is explosive and lasts for 20-40 days. Sanchez et al. have reported that temperature, rainfall, relative humidity and wind speed influence the airborne *P. acerifoli* pollen concentrations in the Iberian Peninsula,[1] and high concentrations of *P. acerifoli* pollen are detected

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during the pollination season in some areas of Spain.[2]

P. acerifolia pollen has been studied widely, particularly in Western European cities in the previous 10 years.[3-7] Three major allergens have been identified from *P. acerifolia* pollen extract.[3, 5, 6] Pla a 1, a non-glycosylated 18 kDa protein, belongs to a family of invertase inhibitors.[4] Pla a 2 is a glycoprotein (polygalacturonase) with a molecular weight of 43 kDa.[5] Polygalacturonases catalyze the degradation of polymeric g, which is a major component of pectin in plant cell walls.[8] Pla a 3, a 9-10 kDa protein, belongs to non-specific lipid-transfer proteins and is related to food allergies.[6] Lipid-transfer proteins have been identified as major allergens in fruits such as peaches and cherries.[9, 10]

Biochemical knowledge regarding allergens improves strategies for purification and characterization and helps to explain the relationships between protein structures and allergenic activity.[11] We describe the purification, immunodetection and mass spectrometry analyses of allergens from *P. acerifoli* pollen. Our results indicate that the pollen of *P. acerifolia* might play an important role in allergic diseases in Shanghai.

MATERIALS AND METHODS

Patient's Sera

The sera samples used in this study were collected in mid to late April from 41 untreated patients exhibiting pollinosis with seasonal rhinitis and/or bronchial asthma. Their serum total IgE levels were determined by the immune scatter turbidity method in the Huashan Hospital laboratory. A pool of sera from nonallergic individuals was used as negative control. The sera samples were kept at -28 °C.

Purification of Pla a 1 and Pla a 2

Pollen grains from *P. acerifolia* were obtained during the flowering season (typically mid to late April in Shanghai) from *P. acerifolia* trees planted in the Jing'an District. The purification method of Pla a 1 and Pla a 2 were as described previously.[3,5] A crude extract of this pollen was prepared after degreasing in ether and soaking in coca's solution (0.5% NaCl, 0.275% $NaHCO_3$) for 48 hours. Then, the extract was fractionated on a HiTrap SP column[3,5] (GE, Uppsala, Sweden) equilibrated with 20 mM phosphate buffer with pH of 6.5 using a step gradient of 0.1, 0.3, 0.5 M NaCl. The protein content of each extract was determined via the Bradford method. Active fractions determined by ELISA incubated with patient (No. 34) serum that reacted exclusively to the crude pollen extract were pooled, dialyzed, and concentrated using Vivaspin 6 MWCO 3,000 d Sample Concentrators (Vivascience, Germany) and kept at -18 °C. The fractions were then applied to a Superdex G 75 column equilibrated with 20 mM PBS, pH 7.0. The active fractions were pooled, concentrated, and kept at -18 °C.

ELISA and Immunoblotting

The crude extract proteins were processed in reducing conditions using β-mercaptoethanol and separated on 15% polyacrylamide gels by SDS-PAGE. The protein bands were visualized with Coomassie Brilliant blue R250. For the immunodetection, the proteins were transferred to PVDF membranes and stained with Ponceau S, and then they were blocked by 5% BSA for 2 hours. The membranes were probed with the sera of the patients with allergic disease individually (diluted 1:10, 4°C, overnight). A pool of sera from nonallergic individuals was used as a negative control. To detect the reactive IgE antibodies, a horseradish peroxidase-labeled mouse monoclonal anti-human IgE secondary antibody (Serotec, Raleigh, NC, USA) was used (diluted 1:1000, 37°C, 1 hour). The signal was detected and visualized using the ECL Wwestern blotting reagent (Pierce, US). The films were scanned using a Luminescent Image Analyzer LAS4000 (Fujifilm, Mishima, Japan).

Quantification of the IgE levels in the sera of all the patients was determined via ELISA. The plates were coated with purified protein or crude extract, blocked in 5% BSA and incubated with all the individual allergic sera (diluted 1:10, 37°C, 1.5 h). The sera from the non-allergic subjects was used as a negative control. Bound IgE antibodies were detected using the identical mAb used for the immunoblotting, as described above. The signal was detected using the OPD-H_2O_2 (Amresco, US) substrate and the OD was read at 492 nm (Labsystems Dragon Wellscan MK-3, Finland). Optical density of more than 2 times the medium values of the negative control was considered to be positive. The experiments were performed in duplicate.

In-gel Proteolytic Digestion and Mass Spectrometry

The protein spots were excised from a fresh CBB-stained gel and plated into a 96-well microtiter plate.

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The excised spots were destained twice with 60 µl of 50 mM NH_4HCO_3 and 50% acetonitrile and dried twice with 60 µl of acetonitrile. The dried gel segments were subsequently incubated in an ice-cold digestion solution (trypsin 12.5 ng/µl and 20 mM NH_4HCO_3) for 20 min and then transferred into a 37°C incubator for digestion overnight. The peptides were collected from the supernatant after 2 extractions in 60 µl extract solution (5% formic acid in 50% acetonitrile).

The peptide solution was dried under N_2. The 0.8-µl matrix solution (5 mg/ml α-cyano-4-hydroxy-cinnamic acid diluted in 0.1% TFA, 50% ACN) was dissolved by repeated pipetting. The mixture was subsequently spotted on a MALDI target plate (Applied Biosystems). The MS analysis of the peptides was performed on an ABI 4700 TOF-TOF Proteomics Analyzer (Applied Biosystems). The UV laser was operated at a repetition rate of 200 Hz with a wavelength of 355 nm. The accelerated voltage was operated at 20 kV, and the mass resolution was maximized at 1500 Da. Myoglobin digested with trypsin was used to calibrate the instrument. The spectra were processed using 4700 ExploreTM Software (Applied Biosystems) in the default mode. The data were searched using GPS Explorer (V3.6) and the Mascot search engine (http://www.matrixscience.com/). The search parameters included the database NCBInr, green plant taxonomy, protein molecular masses ranging from 700 to 3200 Da, and trypsin digestion with one missing cleavage. An MS tolerance of 100 ppm and an MS/MS tolerance of 0.6 Da were used. The proteins with a score greater than 71 were considered to be significant (p<0.05).

Sequence Analysis of Pla a 1 and Pla a 2

The Pla a 1 and Pla a 2 sequences were obtained from Swissprot (http://www.expasy.ch/sprot/). The homologues of Pla a 1 and Pla a 2 and their accession numbers were obtained using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the sequence information was acquired using Batch Entrez (http://www.ncbi.nlm.nih.gov/sites/batchentrez?db=Nucleotide). The evolutionary trees were drawn with Mega 4.0 software (neighbor-joining, bootstrap 1000). The protein sequences were analyzed for the Pfam matches online (http://pfam.sanger.ac.uk/).

RESULTS

Isolation of *P. acerifolia* Pollen Allergen

Crude pollen extract was purified using cation exchange chromatography (Figure 1). Most of the proteins that were bound non-specifically were removed in the 0.1 M NaCl washing step. The fraction most enriched in the IgE antigens was step-eluted off the HiTrap-SP by 0.3 M NaCl (Figure 1A). The 43 and 18 kDa proteins were separated using Superdex G 75 column (Figure 1B).

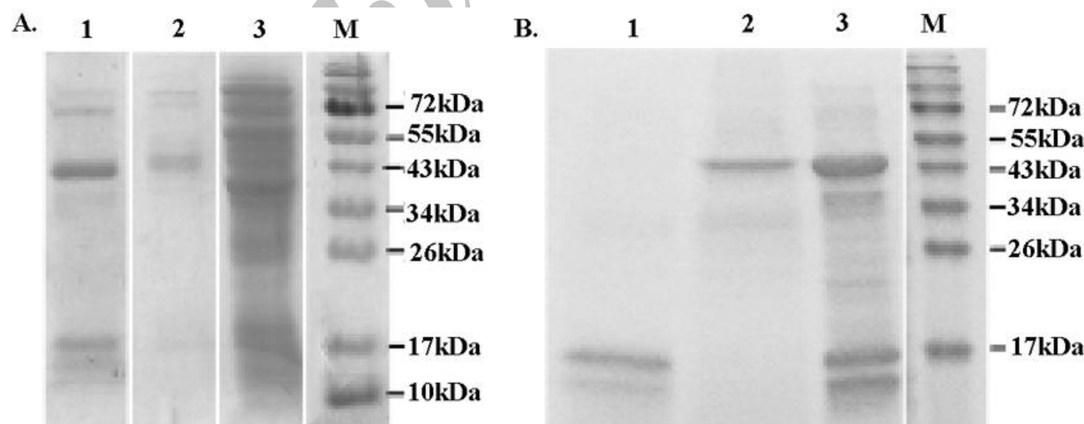

Figure 1. Purification of *P. acerifolia* pollen allergen. (A) Coomassie-stained SDS-PAGE: the protein fraction was purified by a SP column. Lane 1 was the active fraction from the 0.3 M NaCl washing step; lane 2 was from the 0.1 M NaCl washing step; lane 3 was the crude pollen extract. (B) Coomassie-stained SDS-PAGE: the pooled fractions after enrichment by the SP column (lane 3) and the Superdex G 75 column (lane 1-2). Lane M was the pre-stained protein marker. (Fermentas, Inc., Ontario, Canada)

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Immunologic Assays

The immunological activity of the fractions was analyzed by ELISA and immunoblotting (Figure 2). The ELISA results indicated that 12% (5/41) of the subjects were sensitive to Pla a 1 and 9% (4/41) were sensitive to Pla a 2 (Table 1). The serum samples from three patients (No.16, No.34, No.41) recognized 3 specific bands in the crude pollen extract sample. The 43 kDa band signal was stronger than the 18 and 16 kDa band signals (Figure 2).

Table 1. The clinical data of patients and total IgE results of the sera

Patient no.	Sex	Age (y)	Rhinitis	Asthma	Total IgE (ng/ml)	Crude extract	Pla a 1	Pla a 2
1	F	32	+	+	1416	-	-	-
2	M	45	-	-	103	-	-	-
3	M	25	+	+	216	-	-	-
4	M	28	+	+	403.2	-	-	-
5	M	57	+	+	2865	-	-	-
6	F	54	-	+	119	-	-	-
7	M	31	+	+	372	-	-	+
8	F	65	-	+	521	-	-	-
9	F	67	-	-	163	-	-	-
10	M	33	+	+	981	-	-	-
11	F	23	-	+	2856	-	-	-
12	F	46	-	+	152.8	-	-	-
13	F	60	-	-	123	-	-	-
14	M	27	+	+	530	+	+	-
15	M	31	-	+	2640	+	-	-
16	F	38	+	+	2856	+	-	+
17	M	30	+	+	300	-	-	-
18	F	25	-	-	210	-	-	-
19	F	32	+	+	360	+	+	-
20	M	60	+	+	1800	+	+	-
21	F	30	+	+	554.8	+	-	+
22	M	29	+	+	1084	-	-	-
23	F	39	+	+	1010	-	-	-
24	F	40	+	+	656	-	-	-
25	M	37	+	+	268	-	-	-
26	M	23	+	+	333	-	-	-
27	F	34	+	+	50.6	-	-	-
28	M	26	+	+	2856	-	-	-
29	M	44	+	+	2856	-	-	-
30	F	30	-	+	83	-	-	-
31	M	39	+	+	156	-	-	-
32	F	34	+	+	43	-	-	-
33	F	39	+	+	106	-	-	-
34	F	36	+	+	41	+	+	+
35	F	24	+	+	1050	-	-	-
36	M	22	+	+	98	-	-	-
37	F	17	+	+	240.6	-	-	-
38	F	44	-	-	48	-	-	-
39	F	61	-	+	276	-	-	-
40	F	64	-	-	146	-	-	-
41	M	47	+	-	354	+	+	-

The normal reference value of the total IgE was <240 ng/ml.

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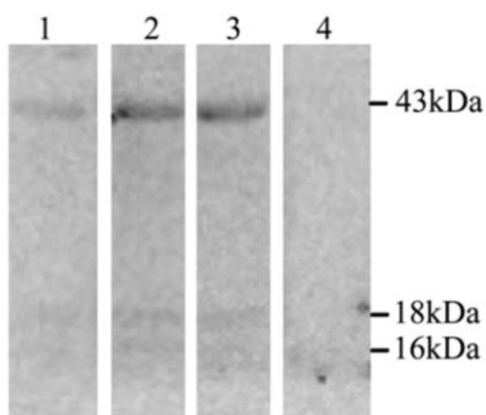

Figure 2. Immunoanalysis of *P. acerifolia* pollen allergen.

The crude pollen extract was separated by SDS-PAGE and blotted onto a PVDF membrane. Lane 1-3 were the membranes with strong signals that were incubated with the serum samples from three patients (No.16, No.34 and No.41), respectively. Lane 4 was incubated with a pool of sera from nonallergic individuals.

Mass Spectrometry Analysis

Figure 3 showed the results from the mass spectrometry analysis of the purified allergens. The data were analyzed using GPS Explorer (V3.6) and the MASCOT search engine (Figure 3 and Table 2). The peptide assignments were displayed in Table 2. The MS/MS generated peptide mass fingerprints (PMF) confirmed the identities of the 18 kDa and 43 kDa proteins as Pla a 1 (Figure 3 A-B) and Pla a 2 (Figure 3 C-D), respectively. The 16 kDa protein had a score of 82 with a predicted protein (gi 224149933).

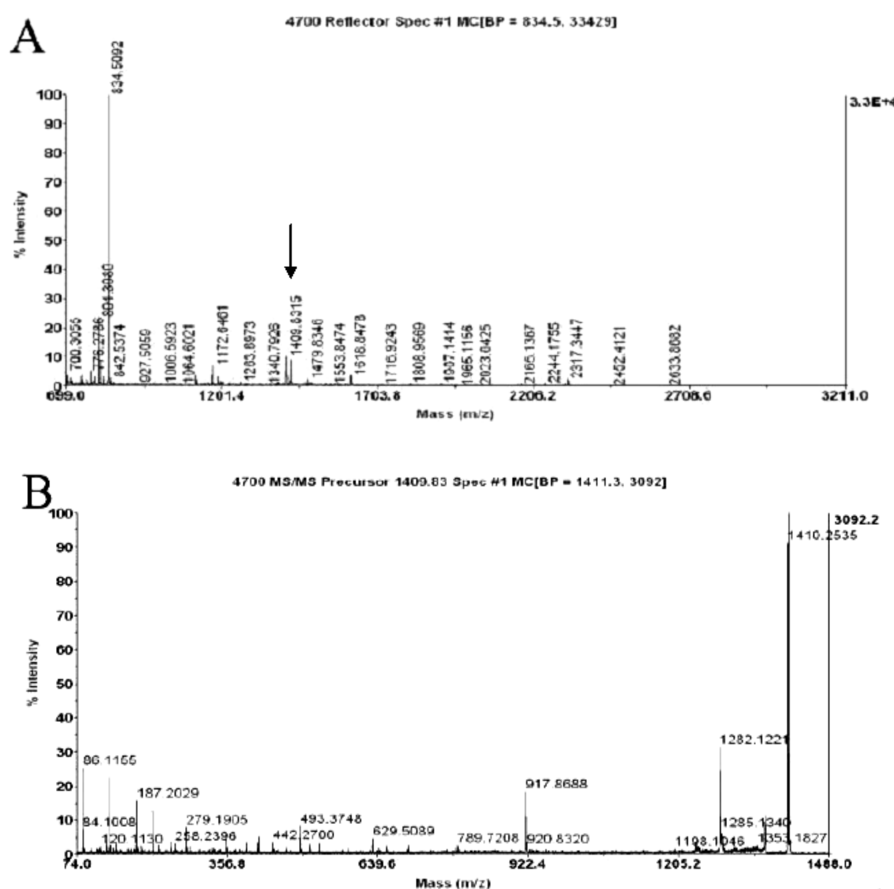

Figure 3. Mass spectrometry analysis of the purified allergens.
(A) The PMF mass spectrum of the 18 kDa protein, and the arrow indicated the peptide that was detected at m/z 1409.83;
(B) the MS/MS spectrum of 1409.83 m/z;

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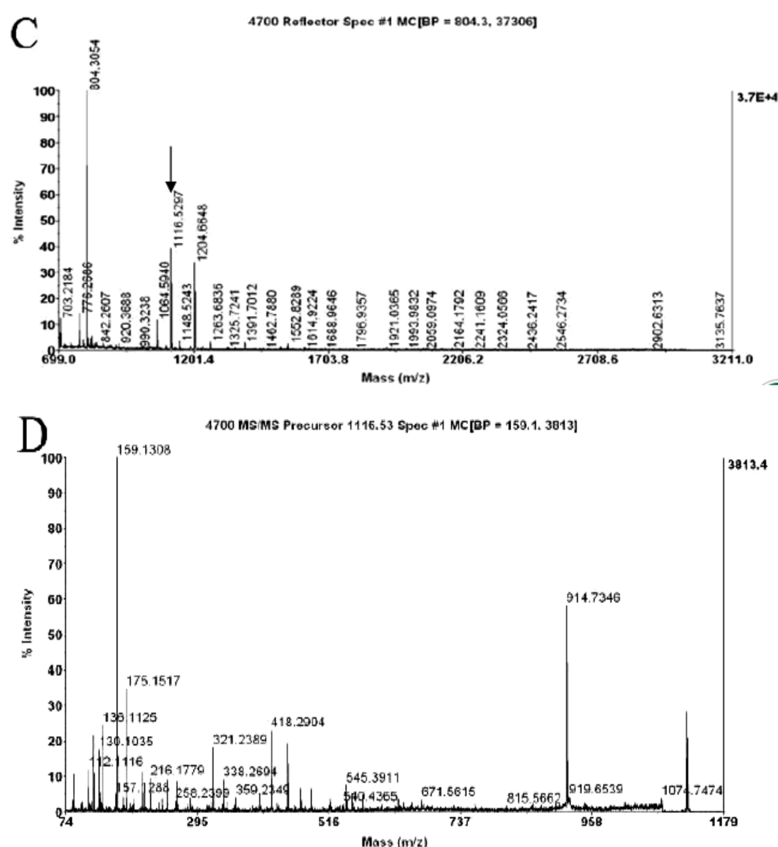

Figure 3. Mass spectrometry analysis of the purified allergens.
(C) the PMF mass spectrum of the 43 kDa protein, and the arrow indicated the peptides that were detected at m/z 1116.53; (D) the MS/MS spectrum of 1116.53 m/z. PMF: peptide mass fingerprinting.

Table 2. Mass spectrum analysis of allergens purified from *P. acerifolia* pollen.

MW of allergen	Protein name	Mass	Protein score	Species	Accession key	Observed mass	Sequence
18 kDa	Pollen allergen Pla a 1	19270	167	*Platanus acerifolia*	gi 29839547	834.5092	^{79}IQTFIGR^{85}
						1194.6375	^{111}SSVQEAIADFK^{121}
						1409.8311	^{111}SSVQEAIADFKSK^{123}
						1535.8984	^{162}DYVQLTAISLAITK^{175}
						1907.1414	^{159}ENKDYVQLTAISLAITK^{175}
						2317.342	^{56}SHTADLQGLGVISANLAIQHGSK^{78}
43 kDa	Pollen allergen Pla a 2	39740	207	*Platanus acerifolia*	gi 51316214	812.4285	^{126}HAAMNLR^{132}
						1051.5354	^{233}YNNEKEVR^{240}
						1064.5924	^{341}IGEINLSYR^{349}
						1075.6432	^{66}IGFQIDGVVK^{75}
						1116.5295	^{85}SDGWVSFYR^{93}
						1204.6643	^{308}LSNINFNNIR^{317}
						1263.6836	^{133}FDFLKHAMVR^{142}
						1391.7004	^{83}FKSDGWVSFYR^{93}
						1445.8737	^{306}IKLSNINFNNIR^{317}
						1462.791	^{126}HAAMNLRFDFLK^{137}

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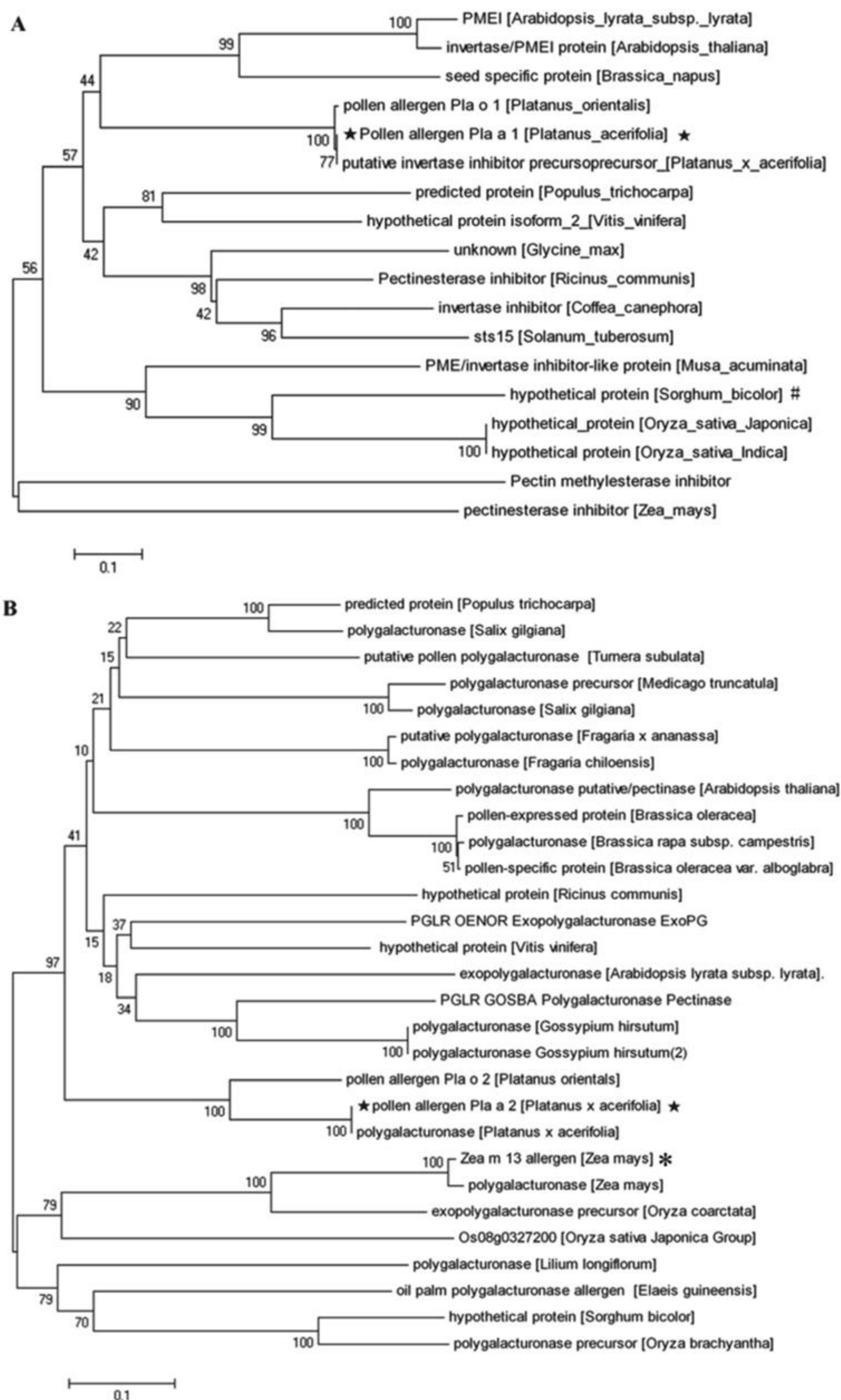

Figure 4. The evolutionary trees of Pla a 1 (A) and Pla a 2 (B).
⋆Platanus pollen allergen; **Zea mays* pollen allergen; # Hypothetical protein from *Sorghum bicolor*

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The Evolutionary Analysis of Pla a 1 and Pla a 2

Figure 4 showed the evolutionary relationships among the pollen allergens from various species. They originated from one common ancestor and mutated into different taxa. Pla a 1 belonged to the PMEI (Plant invertase/pectin methylesterase inhibitor, PF04043) protein family. Pla o 1, as the main allergen of *Platanus orientalis* pollen (the Oriental plane tree), was a homolog of Pla a 1 (Figure 4A). Pla a 1 shared a 97.65% sequence with Pla o 1 and completely shared the identical sequence with the putative invertase inhibitor precursor (Figure 5A). Most of the proteins in this evolutionary tree were invertase inhibitors or related proteins.

Pla a 2 and Pla o 2 belonged to the glycosyl hydrolase family of 28 members (PF00295), and this family had the following additional allergens including four in Cupressaceae and one in Poaceae (according to the internet research): Cry j 2 (*Cryptomeria japonica*), Jun a 2 (*Juniperus ashei*), Cup a 2 (*Cupressus arizonica*), Cha o 2 (*Chamaecyparis obtusa*) and Zea m 13 (*Zea mays*, Figure 4.B).[12] Pla a 2 had a 78.78% similar sequence with Pla o 2 and shared completely the identical sequence with polygalacturonase (Fig 5B). Most of the proteins in the tree were ploygalacturonase.

A

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pollen allergen Pla o 1                MKLSFSLCIFFL---------ISADIVQGTCKKVAQRSPNVNYDFCVKSLGADPKSHSAD
pollen allergen Pla a 1                MKLSFSLCIFFFNLLLLLQAVISADIVQGTCKKVAQRSPNVNYDFCVKSLGADPKSHTAD
putative invertase inhibitor precursor MKLSFSLCIFFFNLLLLLQAVISADIVQGTCKKVAQRSPNVNYDFCVKSLGADPKSHTAD
                                       ***********:         ***********************************:**

pollen allergen Pla o 1                LQGLGVISANLAIQQGSKIQTFIGRILKSKVDPALKKYLNDCVGLYADAKSSVQEAIADF
pollen allergen Pla a 1                LQGLGVISANLAIQHGSKIQTFIGRILKSKVDPALKKYLNDCVGLYADAKSSVQEAIADF
putative invertase inhibitor precursor LQGLGVISANLAIQHGSKIQTFIGRILKSKVDPALKKYLNDCVGLYADAKSSVQEAIADF
                                       **************:*********************************************

pollen allergen Pla o 1                KSKDYASANVKMSAALDDSVTCEDGFKEKKGIASPVTKENKDYVQLTAISLAITKLLGA
pollen allergen Pla a 1                KSKDYASANVKMSAALDDSVTCEDGFKEKKGIVSPVTKENKDYVQLTAISLAITKLLGA
putative invertase inhibitor precursor KSKDYASANVKMSAALDDSVTCEDGFKEKKGIVSPVTKENKDYVQLTAISLAITKLLGA
                                       ********************************.**************************
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B

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pollen allergen Pla o 2   MRGVQSSGGTFNVNDYGAKGSGDISQAVMKAWEAACASPGPSTVLIPTGNYIMGEVLLEG
pollen allergen Pla a 2   -RGVQSSGSVFNVNDYGAKGAGDISQAVMKAWKAACASQGPSTVLIPKGNYNMGEVAMQG
polygalacturonase         -RGVQSSGSVFNVNDYGAKGAGDISQAVMKAWKAACASQGPSTVLIPKGNYNMGEVAMQG
                           *******..**********:***********:***** ********.*** **** ::*

pollen allergen Pla o 2   PCKGSKIGFQLDGVVKAPADVSAFKSEGWVVFNHVDGLTVSGKGTFDGQGQKAWAANNCD
pollen allergen Pla a 2   PCKGSKIGFQIDGVVKAPADPSKFKSDGWVSFYRIDGLTVSGTGTLDGQGQTAWAKNNCD
polygalacturonase         PCKGSKIGFQIDGVVKAPADPSKFKSDGWVSFYRIDGLTVSGTGTLDGQGQTAWAKNNCD
                          **********:********* * ***:*** * ::*******.**:*****.*** ****

pollen allergen Pla o 2   KDENCNRPPMNIRFNFLKNAVVRDITSMNSKMFHINVLECDNISFQHVTISAPGTSINTD
pollen allergen Pla a 2   KNPNCKHAAMNLRFDFLKHAMVRDITSLNSKMFHINVLECEDITFQHVTVTAPGTSINTD
polygalacturonase         KNPNCKHAAMNLRFDFLKHAMVRDITSLNSKMFHINVLECEDITFQHVTVTAPGTSINTD
                          *: **::   **:**:***.*:******:************::*:*****::*********

pollen allergen Pla o 2   GIHIGLSRGVTITDTNIATGDDCVSIGPGSQNVTVTKVNCGPGHGISVGSLGKYKDEKDV
pollen allergen Pla a 2   GIHVGISKGVTITNTKIATGDDCISIGPGSQNVTITQVNCGPGHGISIGSLGRYNNEKEV
polygalacturonase         GIHVGISKGVTITNTKIATGDDCISIGPGSQNVTITQVNCGPGHGISIGSLGRYNNEKEV
                          ***:*:*:*****:*:*******:**********:*:*********:****:*::**:*

pollen allergen Pla o 2   RGITVTGCTFTGTSNGVRVKTWPDSPPGVATDMAFEDLTMKNVQNPVILDQEYCPYGQCS
pollen allergen Pla a 2   RGITVKGCTFSGTMNGVRVKTWPNSPPGAATDLTFQDLTMNNVQNPVILDQEYCPYGQCS
polygalacturonase         RGITVKGCTFSGTMNGVRVKTWPNSPPGAATDLTFQDLTMNNVQNPVILDQEYCPYGQCS
                          *****.****:** *********:****.***::*:****:*******************

pollen allergen Pla o 2   LKAPSRVKLSNIKFNNIRGTSSGPDAIVIACSHGFPCSNLEIGEINLALHAAGAPANSTC
pollen allergen Pla a 2   RQAPSRIKLSNINFNNIRGTSTGKVAVVIACSHGMPCSNMKIGEINLSYRGAGGPATSTC
polygalacturonase         RQAPSRIKLSNINFNNIRGTSTGKVAVVIACSHGMPCSNMKIGEINLSYRGAGGPATSTC
                           :****:*****:********:*  *:*******:****::*******: :.**.**.***

pollen allergen Pla o 2   TNAKPIFSGKQVPAIKCA
pollen allergen Pla a 2   SNVKPTFSGKQVPAIKCA
polygalacturonase         SNVKPTFSGKQVPAIKCA
                          :*.** ************
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Figure 5. The multiple sequence alignment for Pla a 1 (A) and Pla a 2 (B)

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DISCUSSION

Shanghai is located in the middle and lower Yangtze valley plain. This region has a subtropical monsoon climate suitable for plant growth that leads to extensive plant pollen in the air year round. Our predecessors, Wang MK et al., have documented the prevalence of different types of common allergens in Shanghai and East China since the 1960s.[13] Several types of inhaled pollen allergens have been discovered, including pollen from *P. acerifolia* (London plane tree), *Humulus scandens*, *Chenopodium album* (lamb's-quarters), *Broussonetia papyrifera* (paper mulberry), and *Artemisia annua* (sweet wormwood). In mid to late spring, a large amount of *P. acerifolia* pollen is detected in Shanghai. This pollen comprised the second largest amount of the total yearly pollen in Shanghai, reaching 13.8% of the total pollen in 2009.[14] The occurrence , development and subsidence of clinical symptoms are closely related to the spread of allergenic pollen.[15] The causes of the seasonal allergies in patients during different seasons are inconsistent, and different conclusions have been reached regarding the allergic pollen in various areas. *P. acerifolia* pollen has been widely studied, particularly in Western European cities during the last 10 years.[3-7] However, few studies have been reported regarding these important airborne pollen allergens in Shanghai in the previous decade. We focused on the identification and immunological activities of these pollen allergens in the Shanghai area. We used mass spectrometry to identify that the purified 43 kDa protein and 18 kDa protein from *P. acerifolia* pollen extract were pollen allergen Pla a 1 and Pla a 2, which have immunological activities with human IgE antibodies.

Asturias JA et al. reported that Pla a 1 was recognized by IgE in serum from 35 of the 42 (83.3%) Platanus-allergic patients studied, and it accounted for 60% of the total IgE-binding capacity of *P. acerifolia* pollen extract.[4] Crude extract from natural pollen grains has been forbidden for skin prick testing (SPT) or immunotherapy in China since 2000. Thus, our research does not include SPT. Our results showed that 12% (5/41) of the patients have serum in which IgE recognized pla a 1. According to the research data in our lab during the 1990s (data not published), thousands of patients underwent SPT tests, whereas Platanus pollen extract reached an approximate 7% positive reaction rate in the pollen allergic patients.

The allergen shares 43% sequence identity with a grape invertase inhibitor; however, it has no known physiological function.[4] The PMEI domains contain approximately 200 amino acids and are characterized by four conserved cysteine residues. This domain inhibits pectinesterase/pectin methylesterases (PMEs) and invertases via formation of a non-covalent 1:1 complex.[16] Pollen allergens are restricted to a few protein families and show distinct patterns of species distribution. It appears that except for two allergens from the Platanaceae plant, no allergen belongs to the invertase inhibitor family.[17]

Pla a 2 is a 43 kDa protein with polygalacturonase (PG) activity.[5] Polygalacturonase catalyzes the random hydrolysis of 1,4-alpha-D-galactosiduronic linkages in pectate and other galacturonans.[18] The functional roles of PG in pollen tubes might be to act on its own cell wall and facilitate growth and to degrade the walls of stylar cells to allow penetration of the pollen tube. The cross reactivity observed among *P. acerifolia* pollen and food allergies is consistent with the high degree of conservation among PGs.[5]

Tandem MS (MS/MS), which has high-throughput capability, was used to obtain the peptide mass fingerprint. Because a single IgE reactive band might contain more than one protein and 1-D immunoblotting could not show sensitization to individual allergens, essential purification processes such as ion-exchanging and gel filtration could not be omitted.[19] It is hypothesized that two-dimensional electrophoresis (2-DE) and immunoblotting, as high-resolution separation technology, might be more efficient in protein identification and could be helpful in improving the development of pollen allergen research.

Mass spectrometry and analysis is highly relied on for obtaining the data that are available on the internet, which indicates that in some relatively unknown species, particularly those grown in developing countries, few useful results could be obtained. In spite of this finding, mass spectrometry, with SDS-PAGE and trypsin digestion, could reduce traditional laborious experimental processes such as repeated full-length amino acid sequencing and alignment and is shown to be a useful and powerful tool in the rapid identification of pollen proteins.

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REFERENCES

1. Sánchez-Reyes E, de la Cruz DR, Sanchis-Merino ME, and Sánchez-Sánchez.J. First results of Platanus pollen airborne content in the middle-west of the Iberian Peninsula. Aerobiologia. 2009; 25(4):209-15.
2. Subiza J, Cabrera M, Valdivieso R, Subiza JL, Jerez M, Jimenez JA, et al. Seasonal asthma caused by airborne Platanus pollen. Clin Exp Allergy 1994; 24(12):1123-9.
3. Asturias JA, Ibarrola I, Bartolome B, Ojeda I, Malet A, Martinez A. Purification and characterization of Pla a 1, a major allergen from Platanus acerifolia pollen. Allergy 2002; 57(3):221-7.
4. Asturias JA, Ibarrola I, Eraso E, Arilla MC, Martinez A. The major Platanus acerifolia pollen allergen Pla a 1 has sequence homology to invertase inhibitors. Clin Exp Allergy 2003; 33(7):978-85.
5. Ibarrola I, Arilla MC, Martinez A, Asturias JA. Identification of a polygalacturonase as a major allergen (Pla a 2) from Platanus acerifolia pollen. J Allergy Clin Immunol 2004; 113(6):1185-91.
6. Lauer I, Miguel-Moncin MS, Abel T, Foetisch K, Hartz C, Fortunato D, et al. Identification of a plane pollen lipid transfer protein (Pla a 3) and its immunological relation to the peach lipid-transfer protein, Pru p 3. Clin Exp Allergy 2007; 37(2):261-9.
7. Fernandez-Gonzalez D, Gonzalez-Parrado Z, Vega-Maray AM, Valencia-Barrera RM, Camazon-Izquierdo B, De Nuntiis P, et al. Platanus pollen allergen, Pla a 1: quantification in the atmosphere and influence on a sensitizing population. Clin Exp Allergy 2010; 40(11):1701-8.
8. Hadfield KA Bennett AB. Polygalacturonases: Many genes in search of a function. Plant Physiology 1998; 117(2):337-43.
9. Pastorello EA, Farioli L, Pravettoni V, Ortolani C, Ispano M, Monza M, et al. The major allergen of peach (Prunus persica) is a lipid transfer protein. J Allergy Clin Immunol 1999; 103(3 Pt 1):520-6.
10. Scheurer S, Pastorello EA, Wangorsch A, Kastner M, Haustein D, Vieths S. Recombinant allergens Pru av 1 and Pru av 4 and a newly identified lipid transfer protein in the in vitro diagnosis of cherry allergy. J Allergy Clin Immunol 2001; 107(4):724-31.
11. Aalberse RC. Structural biology of allergens. J Allergy Clin Immunol 2000; 106(2):228-38.
12. Pazouki N, Sankian M, Nejadsattari T, Khavari-Nejad RA, Varasteh AR. Oriental plane pollen allergy: identification of allergens and cross-reactivity between relevant species. Allergy Asthma Proc 2008; 29(6):622-8.
13. Chen YZ, Feng ZJ, Wang MK, Chen XJ. Preliminary observations of airborne pollens in Shanghai area in three years from 1962 to 1965. J East China Normal University (Natural Science) 1979; (01):111-20.
14. Huang JH, Wang YF, Shen CL. Monitoring of the airborne pollen grains in Shanghai .J East China Normal University (Natural Science) 2013; (02):56-62.
15. Borish L. Allergic rhinitis: systemic inflammation and implications for management. J Allergy Clin Immunol 2003; 112(6):1021-31.
16. Giovane A, Balestrieri C, Quagliuolo L, Castaldo D, Servillo L. A glycoprotein inhibitor of pectin methylesterase in kiwi fruit. Purification by affinity chromatography and evidence of a ripening-related precursor. Eur J Biochem 1995; 233(3):926-9.
17. Radauer C, Breiteneder H. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. J Allergy Clin Immunol 2006; 117(1):141-7.
18. Huang JH, Schell MA. DNA sequence analysis of pglA and mechanism of export of its polygalacturonase product from Pseudomonas solanacearum. J Bacteriol 1990; 172(7):3879-87.
19. Kukreja N, Singh BP, Arora N, Gaur SN, Sridhara S. Identification of Epicoccum purpurascens allergens by two-dimensional immunoblotting and mass spectrometry. Immunobiology 2008; 213(1):65-73.

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