

Pho d 2, a major allergen from date palm pollen, is a profilin: cloning, sequencing, and immunoglobulin E cross-reactivity with other profilins

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Summary

Background Up to now, some date palm pollen (DPP) allergens have been described but very few data are available about their molecular nature. The aim of this study was to identify and characterize Pho d 2, a major allergen from this pollen.

Methods Sera from 25 patients allergic to DPP were analysed by immunoblotting. Purification of DPP profilin was performed by poly-L-proline affinity chromatography. Profilin-encoding cDNA from DPP was cloned by using a RT-PCR strategy and recombinant allergen was expressed as a non-fusion protein in *Escherichia coli*. Natural and recombinant Pho d 2 were investigated by means of enzyme allergosorbent test to compare the immunologic properties of both allergens and to analyse cross-reactivity with other profilins.

Results A 14.4 kDa protein was identified as a major allergen in DPP extract. Purification, cloning, heterologous expression, and inhibition experiments identified it as profilin (Pho d 2). Pho d 2 comprises 131 amino acids and has high sequence identity with other allergenic food and pollen profilins. The prevalence of specific IgE antibody reactivity to natural Pho d 2 by ELISA was 56% and 64% by skin prick test (SPT). Pho d 2 is an important allergen as it is responsible for more than 70% of the IgE reactivity to the pollen extract. IgE directed against Pho d 2 showed a strong cross-reactivity with other profilins such as those from olive tree and grass pollens.

Conclusion Pho d 2, a 14.4 kDa protein identified as profilin, is a major and relevant allergen in DPP, as confirmed by SPT and thereby may elicit clinical symptoms in sensitized patients.

Keywords IgE cross-reactivity, Pho d 2, *Phoenix dactylifera*, profilin, recombinant allergen, skin prick test

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Introduction

The date palm (*Phoenix dactylifera*) belongs to the Aracaceae Family and is indigenous to the Arabian Peninsula, the Mediterranean, and North Africa countries, parts of India and hotter parts of the USA. Date palm pollen (DPP) has been identified as a potent allergen source [1], with sensitization rates among respiratory patients ranging from 25% in Saudi Arabia [1] to 18.9% in Spain [2], and with higher incidence among residents of rural than of urban communities [3]. Differences of allergenicity of DPP have been reported as a cultivar-specific phenomenon [4, 5]. Sensitization to other species from the genus *Phoenix*, *P. silvestris* and *P. canariensis*, have also been reported [6, 7]. Six allergen components of <12, 14, 28–30, 37–40, 57, and 65–67 kDa binding 60–93% of atopic sera have been identified and named from Pho d 1 to Pho d 6 in the order listed [1, 8], but few data on their biochemical characterization have been

reported. Date fruits are a staple food in the Middle East and an important component in hundred of foods eaten throughout the world. Allergy to date fruits has been reported [9, 10] and sera from both fruit- and pollen-allergic patients recognized common allergens [9]. Recently, cross-reactivities between date pollen allergens and foods implicated in the oral allergy syndrome have been determined [11].

Profilins are ubiquitous cytosolic actin and phosphatidylinositol 4,5-bisphosphate-binding proteins with molecular masses of 12–15 kDa, which plays a crucial role in regulating the activity in the microfilament system and intracellular calcium levels [12–14], and are produced in high amounts, for e.g., after pollen hydration. They are highly conserved proteins, and sequence comparisons among several plant profilins show an identity of at least 75%. Profilins have been identified as minor allergens in several plant species of tree, grass, and weed pollen and in many fruits and vegetables. It is described as a pan-allergen since patients sensitized to profilins characteristically react to a broad range of inhalant and food-related allergen sources [15–18].

In this study, we confirm profilin as a major allergen of *P. dactylifera* pollen and its complete amino acid sequence was deduced from the cDNA sequence. The recombinant

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non-fusion protein was produced in *Escherichia coli*, purified and tested with sera from DPP -allergic patients. Moreover, the cross-reactivity with other profilins was investigated by dose-dependent inhibition experiments.

Materials and methods

Pollen crude extract preparations and profilin purification

Crude extract of pollen from about 10 male trees of *P. dactylifera* (locally collected from Elche, Spain), *Lolium perenne* and *Olea europaea* (both from Polen Mediterráneo, Jaén, Spain) were prepared as previously described [19]. Quantification of profilin content was performed using double-sandwich ELISA as described elsewhere [20].

Profilin purification was accomplished by affinity chromatography in poly-(L-proline)-Sephacrose (PLP-Sephacrose) column [21] on an ÄKTA-prime SystemTM (Amersham Biosciences, Uppsala, Sweden). Protein-containing fractions were pooled, dialysed 3 times within 24 h against buffer containing decreasing amounts of urea (4 M, 2 M, and without urea, respectively) and lyophilized. Protein content of each extract was determined by the Bradford's method (Bio-Rad, Palo Alto, CA, USA) using bovine serum albumin (BSA) as standard.

Patient sera and antiserum

Patients allergic to DPP were diagnosed according to case history, display of symptoms after inhalation of DPP, positive skin prick test (SPT) against a standardized DPP extract (Bial-Aristegui, Bilbao, Spain), and specific IgE against DPP extract (Table 1). Profilin specific rabbit antiserum was prepared as reported in Asturias et al. [22]. Specific IgE were analysed by UniCap System (Pharmacia Diagnostics, Uppsala, Sweden) or enzyme allergosorbent test (Hytec-specific IgE enzyme immunoassay; Hycor Biochemical, Kassel, Germany).

Skin prick test

SPT were performed on the volar side of the forearm with a standardized prick needle (Stallergens, Antony, France), applying 25 µL of DPP profilin. Histamine dihydrochloride (10 mg/mL) was used as positive control, and the glycerol-containing diluent of the prick solution was used as negative control. Tests were performed consecutively with DPP profilin at protein concentration of 4, 40, 400 µg/mL and biologically standardized DPP extract (Bial-Aristegui). A weal size of 7 mm² or greater was regarded as positive.

Table 1. Clinical data of allergic patients used in the study and binding of specific human IgE to DPP extract and purified natural and recombinant Pho d 2

Sera	Age/gender	Symptoms*	SPT† with extracts from			ELISA‡			Immunoblot	
			DPP	Olive tree	<i>L. perenne</i>	DPP extract	nPho d 2	rPho d 2	nPho d 2	rPho d 2
1	22/F	RC	1	3	2	0.470	1.124	1.333	++	+
2	46/F	R	4	3	4	2.649	0.148	0.104	–	–
3	58/F	R, A	3	2	3	0.927	0.096	0.100	–	–
4	65/M	R, A	2	3	4	0.457	1.320	2.589	+	++
5	29/M	R, RC	2	4	3	1.579	2.172	1.417	++	+
6	43/M	RC, A	4	2	4	0.734	0.112	0.100	–	–
7	27/M	R, A	4	4	4	2.853	0.610	0.154	–	–
8	29/M	R, A	4	3	ND	1.570	0.151	0.112	–	–
9	42/F	R, A	3	ND	4	2.348	1.218	0.825	++	+
10	40/M	R, A	4	4	4	2.751	0.190	0.121	+/-	–
11	48/F	RC	4	2	2	0.926	0.250	0.161	+/-	–
12	24/F	RC, A	3	4	4	1.270	0.438	0.321	+/-	+/-
13	37/M	RC, A	2	4	4	1.445	1.149	0.685	++	+
14	42/F	R, A	1	4	4	1.147	0.224	0.105	+/-	+/-
15	41/F	RC	4	4	ND	2.353	0.141	0.148	–	–
16	25/F	RC, A	4	4	4	2.228	0.991	1.207	++	++
17	41/F	RC, A	4	3	2	2.000	0.645	0.559	+	+
18	34/M	RC, A	3	4	2	0.497	1.197	1.964	++	++
19	26/M	RC	4	ND	ND	2.814	0.202	0.108	–	–
20	27/M	RC, A	4	3	4	1.831	0.217	0.112	+/-	+/-
21	39/F	R, A	2	3	3	2.707	0.178	0.091	–	–
22	25/F	RC	4	4	4	2.415	0.120	0.101	–	–
23	42/F	R, A	3	4	3	0.573	1.299	1.609	+	+
24	38/F	RC, A	4	4	4	0.710	1.713	2.236	+	++
25	34/F	RC	2	4	4	2.668	0.416	0.343	+/-	+/-
C	pool	–	–	–	–	0.128	0.083	0.080	–	–

*Symptoms: A, asthma; R, rhinitis; RC, rhinoconjunctivitis.

†SPT area was expressed by comparison with histamine produced wheal (10 mg/mL) as follows: – (<25%), 1 (25–50%), 2 (50–100%), 3 (100–200%), 4 (>200%).

‡Figures represent values of absorbance at 492 nm obtained in ELISA screening. Allergosorbent wells were prepared with either DPP extract or purified Pho d 2 and incubated with individual sera from patients sensitized to DPP. Values lower than three-fold the control were considered negative and marked in italics.

ND, not determine; SPT, skin prick test; DPP, date palm pollen; *L. perenne*, *Lolium perenne*; M, male; F, female.

Immunoblotting

Proteins were analysed by SDS-PAGE under reducing conditions [23] and visualized by Coomassie Blue R250 staining. Separated protein bands were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes [24]. After blocking for 1 h with 5% defatted dry milk in Tris-buffered saline (TBS), membranes were incubated overnight at 4 °C with allergic patients' sera. Human anti-IgE-peroxidase conjugates were detected by the chemiluminescent method as recommended by the manufacturer (ECL-Plus™, Amersham Biosciences). The resulting electrophoretic patterns were evaluated by image analysis in a GS-710 Image Analyser (Bio-Rad).

Inhibition assays

For ELISA inhibition assays, microtiter plates were coated with 2 µg/mL of DPP natural profilin or 40 µg/mL of DPP extract in 0.1 M bicarbonate buffer pH 9.6 and saturated with blocking buffer (phosphate-buffered saline (PBS) supplemented with 0.05% Tween 20 and 1% BSA). Hundred microliters aliquots, in triplicate, of serum pool from DPP-allergic patients (1:2 dilution in blocking buffer), previously incubated with different concentrations of the inhibitor, were subsequently added and incubated for 1 h at 37 °C. Bound complexes were detected by incubation for 1 h at 37 °C with horseradish peroxidase-conjugated mouse anti-human IgE antibody (Dako, Glostrup, Denmark). After three washes, the peroxidase activity was measured adding 200 µL/well of a solution of *o*-phenyldiamine (Sigma, St Louis, MO, USA). After 30 min, colour reaction was stopped by adding 50 µL/well of 3 M H₂SO₄, and the optical density was read at 492 nm.

For immunoblot-inhibition studies, 0.5 mL of 1/2 diluted serum pool from date palm-allergic patients was incubated with 10 µg purified profilin or 10 µg BSA (negative control) overnight at 4 °C. The pre-absorbed serum was then used for immunoblot experiments. Profilins from *O. europaea* and *Mercurialis annua* were obtained as previously described [25, 26].

Cloning of date palm pollen profilin cDNA

Poly(A⁺) mRNA, isolated from 100 mg of *P. dactylifera* pollen by the Quick Prep MicroRNA Purification Kit, was reverse transcribed using the First-Strand cDNA Synthesis Kit (both from Amersham Biosciences). Degenerate oligodeoxynucleotide primers (Sigma-Genosys, Cambridge, UK) for cDNA amplification used were designed according to consensus sequence for plant pollen profilins: PF: 5'-AGAGAATTCCATATGTCGTGGCA(A/G)(A/G)CGTACGT and PR: 5'-AGAAAGCTTTCATTAGAGGTCACCGAGCCTCTC. *Eco*RI, *Nde*I, and *Hind*III restriction sites are marked. Profilin encoding cDNA was amplified by PCR using 5 µL of the products of the first-strand cDNA synthesis and the described degenerate primers. The predominant band was isolated from 2% agarose gels (GeneClean, Bio101, La Jolla, CA, USA) and, after cutting with *Nde*I and *Hind*III, cloned into the expression vector pKN172 [27].

Production of recombinant profilin

Expression of recombinant protein was induced in *E. coli* BL21 (DE3) [28] cells containing the pKN172-derived recombinant plasmids with 0.6 mM isopropyl-thio-β-galactoside for 3 h at 37 °C. Cells were harvested by centrifugation, and the pellet, resuspended in 0.05 M Tris-HCl, pH 7.5, 0.1 M NaCl, was lysed by lysozyme treatment (100 µg/mL, 30 min, 37 °C) and mild sonication. Insoluble material was removed by centrifugation and profilin in the supernatant was purified by PLP-Sepharose chromatography as above described.

Results

Immunoglobulin E-reactivity of sera from allergic patients to *Phoenix dactylifera* pollen

Sera from 25 patients allergic to DPP were individually tested by specific IgE immunodetection after SDS-PAGE of DPP extract carried out under reducing conditions (Fig. 1). IgE-

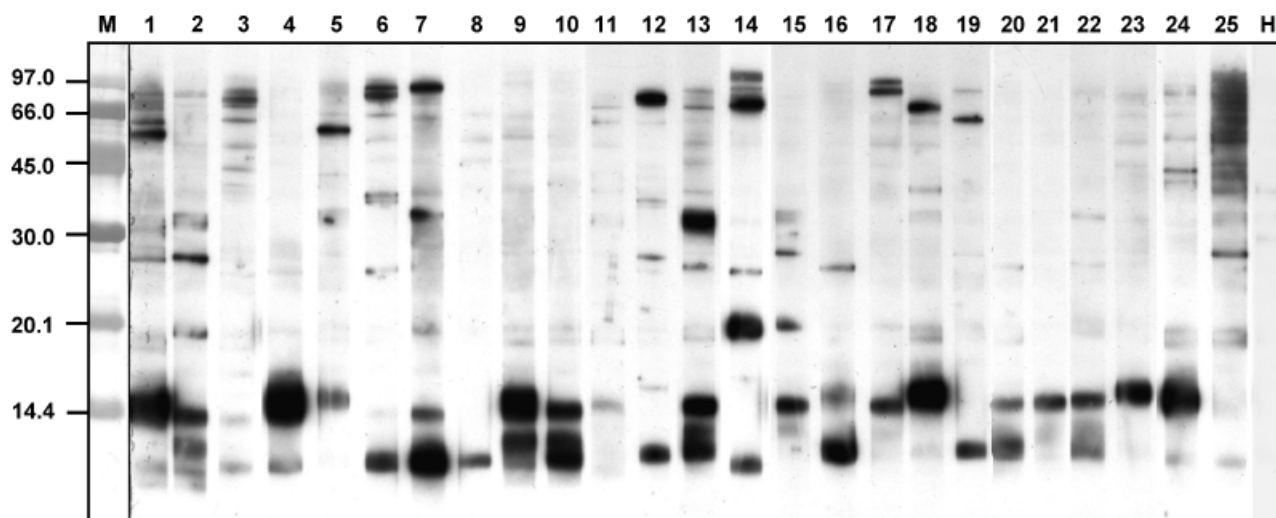


Fig. 1. IgE-reactivity of sera from date palm-allergic patients with *Phoenix dactylifera* pollen allergens separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins transferred onto polyvinylidene difluoride -membranes were probed with sera from date palm-allergic patients (lanes 1–25) and serum pool from healthy individuals (lane H). Molecular mass markers were stained with Amido Black B (lane M).

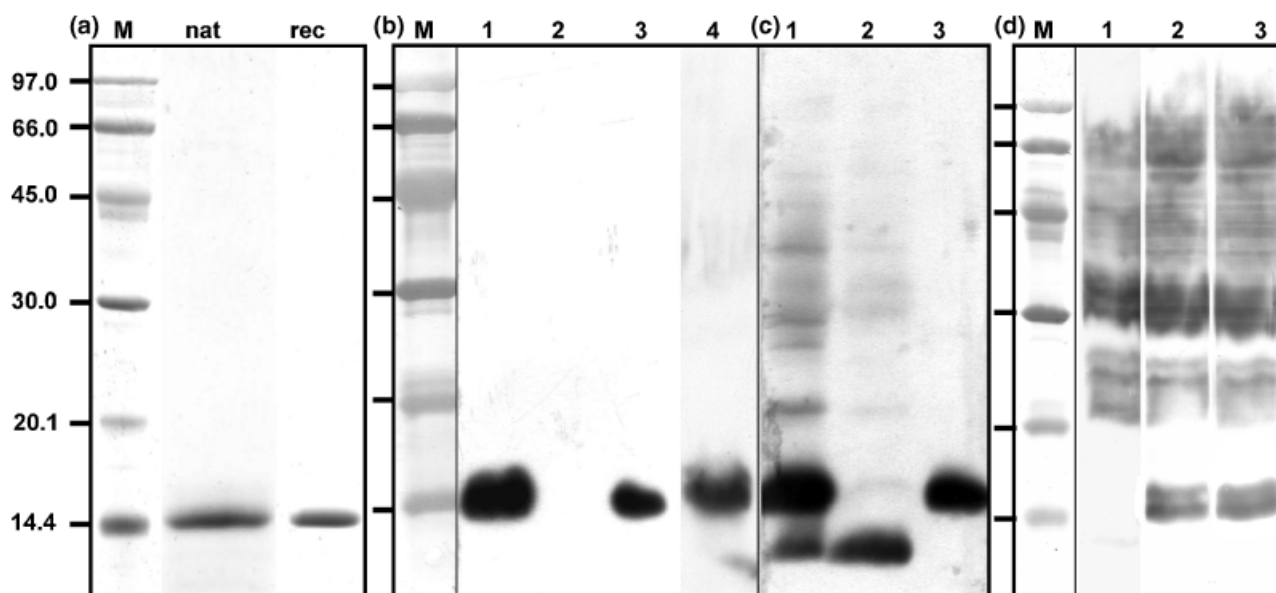


Fig. 2. (a) Coomassie-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis of nPho d 2 (nat) and rPho d 2 (rec). (b, c) Immunoblot of date palm pollen (DPP) extract (lane 1), Pho d 2-depleted DPP extract (lane 2), nPho d 2 (lane 3), and date fruit extract (lane 4) incubated with a rabbit antiserum against profilin (b) or with a pool of sera from DPP-allergic patients (c). (d) IgE-immunoblotting inhibition. A pool of sera from DPP-allergic patients was preabsorbed with 20 µg/mL of nPho d 2 (lane 1), bovine serum albumin (lane 2), and buffer alone (lane 3) and then exposed to polyvinylidene difluoride-blotted date fruit extract.

binding components were detected ranging molecular masses from 9 to 98 kDa. The most relevant IgE-reacting proteins were located at 14–15 kDa, present in 20 out of 25 sera (80%), and at 9 kDa, present in 18 out of 25 sera (72%). Other important IgE-binding components, present in 10–12 out of 25 sera, were detected at 20, 27, 55, 67, and 98 kDa. The 14–15 kDa protein reacted with a rabbit antiserum against profilin (Fig. 2B, lane 1). Date fruit extract showed an IgE-binding protein at 14.4 kDa that corresponded to profilin as it reacted with specific antiserum against profilin and was inhibited by DPP profilin (Fig. 2).

Isolation and immunochemical characterization of natural date palm pollen profilin

Profilin isolation from DPP was performed by affinity chromatography on PLP-Sepharose column. The purified protein showed a single band with an apparent molecular mass of 15.2 kDa and a purity of more than 95% as determined by Coomassie Brilliant Blue-stained SDS-PAGE (Fig. 2A). Rabbit anti-profilin reacted with PLP-purified protein and did not react with profilin-depleted DPP extract (Fig. 2B, lanes 2 and 3), confirming that the purified protein is a profilin and the DPP extract after the purification contained no profilin. Incubation of this profilin-depleted DPP extract with patients' sera showed a faint IgE-reacting band at 14–15 kDa (Fig. 2C, lane 2). To further confirm the identification of the PLP-purified 15 kDa component as profilin, an immunoblot-inhibition assay was performed with natural olive pollen profilin (Ole e 2) as the inhibitor. Ole e 2 was able to completely inhibit IgE-binding to the 15 kDa protein of DPP extract (data not shown). A 14 kDa band in date fruit extract was also identified as profilin based on its reactivity with rabbit antiserum against profilin (Fig. 2B, lane 4). This band reacted with IgE from a pool of sera from DPP allergic

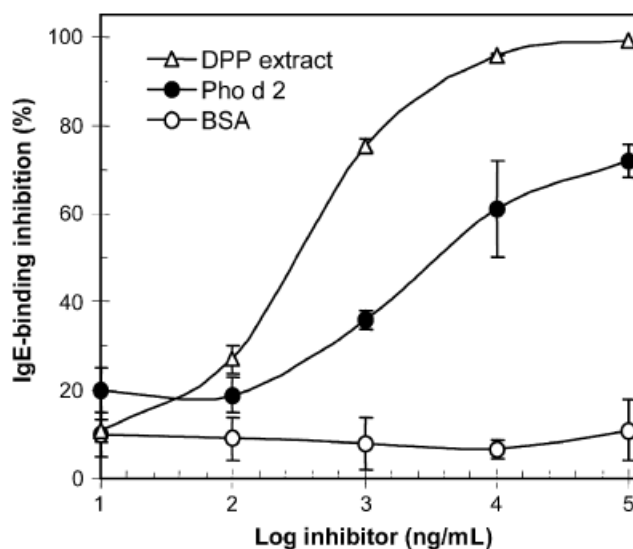


Fig. 3. IgE-ELISA inhibition. The binding of specific human IgE to date palm pollen (DPP) extract was inhibited using nPho d 2, and DPP extract or bovine serum albumin as controls.

patients and this reactivity could be inhibited by purified profilin from DPP extract (Fig. 2D). *P. dactylifera* pollen profilin was named Pho d 2 in accordance with the IUIS/WHO Allergen Subcommittee (<http://www.allergen.org>). Profilin content of DPP extract, estimated by double-sandwich ELISA using Pho d 2 as standard, was 18.7 µg/mg of protein. The IgE-binding activity of Pho d 2 in the *P. dactylifera* extract was estimated by ELISA-inhibition. Purified Pho d 2 inhibited the binding of specific IgE from a pool of patients' sera to the whole extract, up to 73% (Fig. 3), showing the importance of Pho d 2 allergen in the total IgE-binding activity of DPP extract.

Skin prick test

In order to confirm the allergenic activity of Pho d 2, its ability to induce skin prick positive responses was investigated. Skin prick testing with Pho d 2 was positive in 14 out of 22 tested patients (64%) at the highest concentration (400 µg/mL), while at 40 µg/mL, 50% of the patients (11 out of 22) had positive response. The lowest concentration of Pho d 2 (4 µg/mL) was able to elicit positive reactions in only three patients.

Cloning of cDNA, recombinant expression and molecular characterization of profilin from date palm pollen

Amplification of DPP cDNA using PF/PR primers produced a main fragment of approximately 400 bp, with both ends deriving from the degenerate primers, that was cloned in the pKN172 expression vector. DNA sequencing revealed a 393 bp open reading frame encoding a polypeptide of 131 amino acids with a predicted molecular mass of 14.269 Da and a pI value of 4.89. The nucleotide sequence for Pho d 2 has been submitted to the GenBank Database under accession number AJ417566. Comparison of its deduced amino acid sequence using the BLAST program showed high identities (71–87%) with other pollen and plant food profilins: Pyr c 3, 87%; Hev b 8.0201, 86%; Cyn d 12, 83%; Ole e 2, 81%; Phl p 12, 80%; Bet v 2, 79%; and Hel a 2, 71%. Profilins from less related organisms such as *Physarum polycephalum* and human, gave lower homology values, 43% and 31% amino acid identity, respectively.

A non-fusion form of profilin was expressed in *E. coli* BL21 (DE3) using the T7 system. The soluble material, more than 95% expressed profilin, was chromatographed over a PLP-Sepharose affinity column. After elution with 6 M urea in PBS, rPho d 2 showed a single band with an apparent molecular mass of 14.7 kDa and a purity of more than 95%, as determined by Coomassie Brilliant Blue-stained SDS-

PAGE. The yield of purified recombinant profilin was about 5.9 mg/L of bacterial culture.

The capacity of isolated natural and recombinant Pho d 2 to bind specific human IgE after the purification process was evaluated by ELISA in which the purified allergens were adsorbed to the plate. Fourteen out of the 25 sera from DPP-allergic subjects (56%) had specific IgE to nPho d 2 and 12 (48%) to rPho d 2 (Table 1). IgE-reactivity to Pho d 2 was further investigated by immunoblotting experiments using purified natural and recombinant Pho d 2: 16 out of the 25 sera analysed (64%) reacted with the natural profilin and 56% with the recombinant counterpart.

ELISA inhibition studies with pollen extracts and natural and recombinant profilins

Inhibition of IgE-binding to DPP extract and nPho d 2 was performed after pre-incubation of the serum pool from DPP-allergic patients with protein extracts from DPP, *L. perenne* and *O. europaea* pollen. Grass and olive pollen extracts were able to inhibit 50% IgE-binding to DPP extract. Profilins present in these extracts were able to inhibit IgE-binding to nPho d 2 in a dose-dependent manner (Fig. 4). Inhibition of Pho d 2-specific IgE was achieved at 1 µg/mL of DPP extract, in contrast, similar inhibition levels with the other extracts was reached with 1000-fold more protein.

Dose-dependent IgE inhibition experiments were performed by ELISA to investigate the cross-reactivity between nPho d 2 and rPho d 2 and profilins from different sources (Fig. 5). Complete inhibition of IgE-binding to nPho d 2 was observed with nPho d 2 at 10 µg/mL, whereas 88% and 85% reduction of antibody binding were observed with natural pollen profilins from olive and *M. annua* (Fig. 5). Approximately 70% of IgE inhibition was observed after pre-incubation with 10 µg/mL recombinant profilins from DPP and olive pollen. Moreover, using rPho d 2 as solid phase, IgE-binding was almost completely abolished with 1 µg/mL

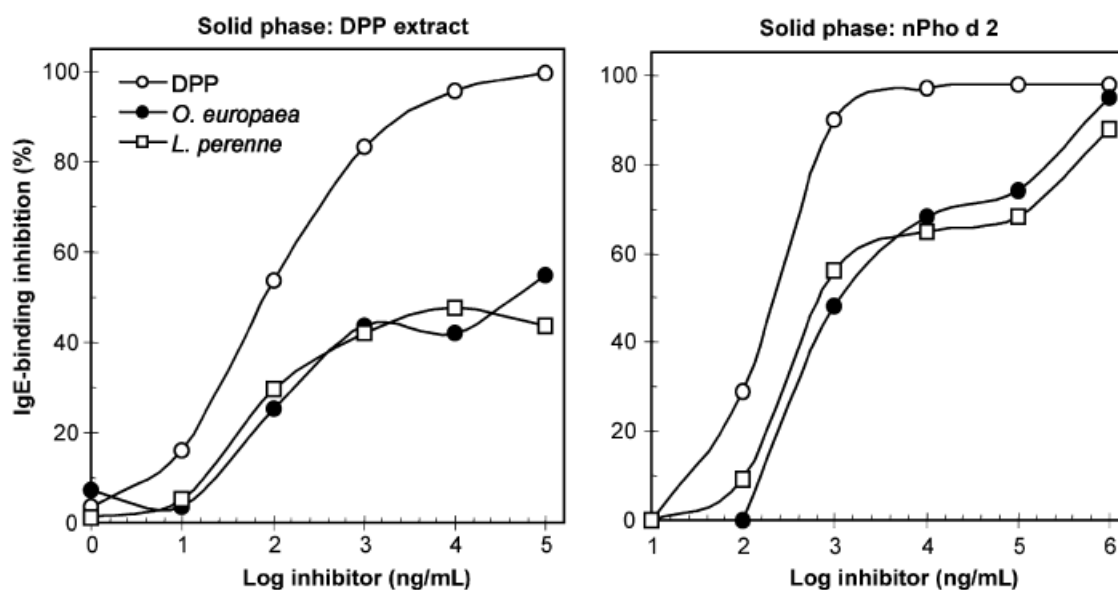


Fig. 4. IgE-ELISA inhibition with natural plant protein extracts. Microtiter plates were coated with 40 µg/mL of date palm pollen (DPP) extract or 2 µg/mL of nPho d 2. A pool of sera from DPP-allergic patients was preincubated with different concentrations of pollen extracts from DPP, *Olea europaea*, and *Lolium perenne*.

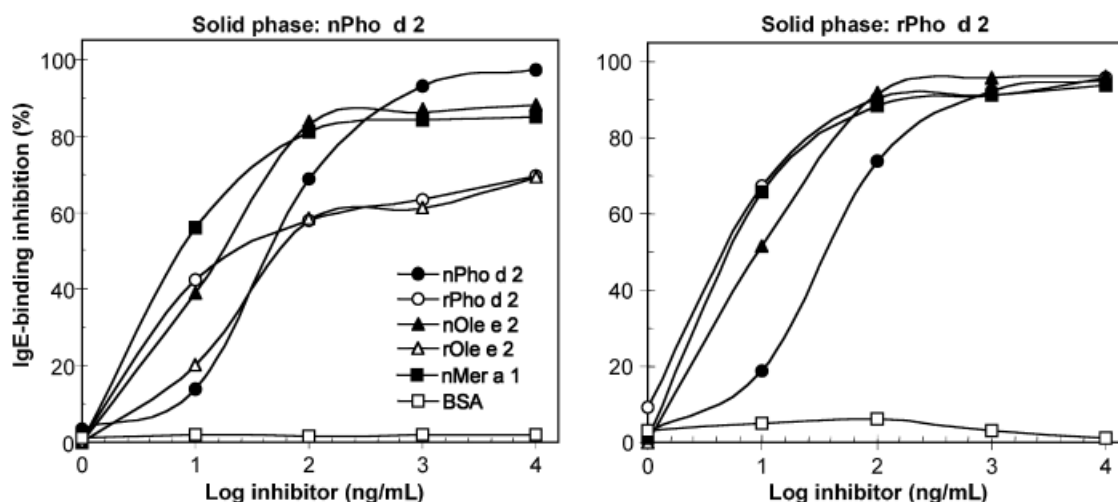


Fig. 5. IgE-ELISA inhibition. Microtiter plates were coated with 2 µg/mL nPho d 2 or rPho d 2. A pool of date palm pollen-allergic patients' sera was preincubated with different concentrations of nPho d 2, rPho d 2, nOle e 2, rOle e 2, nMer a 1, and bovine serum albumin as negative control.

of nPho d 2, rPho d 2, and profilins from olive, and *M. annua* (Fig. 5). ELISA-inhibition assay using rabbit antiserum against profilin showed similar results suggesting that IgG-binding epitopes were also conserved (data not shown).

Discussion

Palm pollen grains are predominant aeroallergens in tropical and subtropical regions and sensitization to this pollen has been shown to represent an important cause of pollinosis in these regions [29]. In some Mediterranean Spanish regions with extensive areas of date palm trees up to 19% positive responses to date palm has been found in pollinic subjects [2]. In the present work, we report the characterization of a major allergen of DPP as profilin, its cloning and heterologous expression.

IgE-reactivity of 25 DPP-allergic patients showed two major IgE-binding components at 14–15 and 9 kDa, and other important IgE-binding proteins on DPP extract (20, 27, 55, 67, and 98 kDa), which are in agreement with some of the allergens previously detected in Saudi Arabian patients (<12, 14, 28–39, 57, and 65–66 kDa) [8]. The molecular mass of the 14–15 kDa band was the same as profilin, a ubiquitous protein known as an important plant panallergen [15]. The identification of Pho d 2 as profilin, reported here, confirms a recent mass spectrometry analysis of a 14 kDa band from date palm which showed sequence homologies with wheat profilin-3 [29]. Isolation of DPP profilin by PLP-chromatography was highly effective and demonstrated that most of the IgE-binding activity of DPP extract is indeed because of Pho d 2 (Fig. 2C). Kwaasi et al. described another 14-kDa allergenic polypeptide from DPP that shares sequence homology to a wound-induced protein of 22.5 kDa and to a pathogenic related protein of 15.7 kDa [29]. The differences in prevalence of purified Pho d 2 and the 14–15 kDa IgE-binding band detected by immunoblotting on sera 4 and 24 (Fig. 1) and the very faint IgE-reaction band of 14 kDa detected after depletion of profilin (Fig. 3C, lane 3) could be because of this allergen.

Our SPT results indicate that DPP profilin is a clinically relevant and major allergen in DPP, which may elicit symptoms in sensitized subjects. Immunological characterization showed that 56% of allergic patients presented sera with a specific IgE-reactivity to DPP profilin, and therefore Pho d 2 is considered a major allergen. These data agree with previous results on date fruit showing that 66% of individual date hypersensitive human sera bound IgE in putative date fruit profilin [11]. The frequency of IgE-reactivity to profilin has been reported to be 20% of all patients allergic to pollen [15], but recent studies showed that profilin is a major allergen in soybean [30], melon [31], *M. annua* pollen [32], and *Chenopodium album* pollen [33]. In DPP allergy, profilin is not only a major allergen but an important allergen as well, as it is responsible for more than 70% of the IgE reactivity of the pollen extract (Fig. 3). Two main aspects could explain this unusual strong sensitization. First, extensive *P. dactylifera* plantations in the area where patients were selected (Elche, Spain) produced from April to May high amounts of DPP that can reach accumulative counts of 3200 grains/m³ with peaks of more than 325 grains/m³ (<http://www.seaic.es>). Second, the profilin content in this DPP extract is 18.7 µg/mg of protein, which is the highest profilin content found among several plant species [20]. These two facts produce a high presence of profilin in the air that could induce strong sensitization in some patients.

ELISA-inhibition experiments showed that IgE-binding of rPho d 2 is very similar but not identical to that of its natural counterpart as the former was able to achieve only 70% of the inhibition produced when nPho d 2 was used. The IgE reactivity of nPho d 2 and its prevalence among DPP-allergic patients was higher than that of rPho d 2 (Table 1). Recombinant profilins are sometimes less reactive than their natural counterparts in ELISA [22, 25, 32] and rPho d 2, prepared in our laboratory, could represent only one of the various isoforms occurring naturally and hence possess only some of the IgE epitopes present in DPP profilin isoforms pool. Furthermore, overproduction of foreign proteins in the *E. coli* cytoplasm often goes together with misfolding of the three dimensional structure of the recombinant proteins.

The specific IgE-binding to profilin in sera from patient 7 seems to be very dependent of protein conformation as they bound to nPho d 2 in ELISA, but no IgE reactivity could be found to rPho d 2 in ELISA and to DPP profilins after denaturing conditions (Table 1). IgE reactivity of the soybean profilin (Gly m 3) is mediated by conformational epitopes and overlapping fragments of rGly m 3 did not show significant reactivity [30].

Profilins are described as panallergens in a wide variety of plant sources [15, 18, 22, 25, 32–38]. To investigate the IgE-cross-reactivity of Pho d 2 with other allergenic pollen profilins, we used a pool of sera from DPP-allergic patients. Natural profilins had the highest inhibition potency whereas recombinant profilins inhibited the IgE reactivity up to 70% at the highest concentration. Cross-inhibition experiments with profilin support the assumption that Pho d 2 shares epitopes with profilins from different botanical origins such as the graminea *L. perenne*, olive tree, and the euphorbiacea *M. annua* (Fig. 4 and 5). The identification of common epitopes among plant profilins needs to be addressed in order to face the development of a putative immunotherapy based on profilin panallergenic epitopes, which could be used in cases of profilin-allergy.

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