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Differential allergen sensitization patterns in common Cypress (*Cupressus sempervirens*) pollen allergy: identification of a novel alkaline allergen

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Highlights

Conclusions:

The use of detergents increases the quality and quantity of extracted proteins in *Cupressus sempervirens* pollen extracts. The present study led to the revelation of at least a novel basic allergen as well as differential allergen sensitization patterns. These novel IgE reactive components may subsequently be applied to expand the panel of well-defined cypress pollen molecules for a more efficient allergen-based diagnosis and therapy.

Cypress pollen allergy: a worldwide pollinosis

The common cypress (*C. sempervirens,* Cs) is the major species growing around the Mediterranean basin and its pollen represents the principal cause of late winter-early spring allergy. Up to now, two Cs allergens have been well characterized:

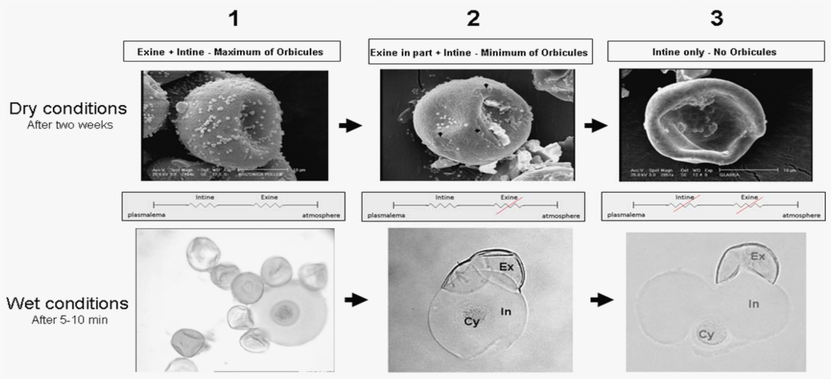
Cup s 1, a **45 kDa** protein within the pectase-lyase family and **Cup s 3**, a thaumatin-like protein of **34 kDa** .

Objective

Till now, most of the reported investigations on pollen molecular allergens were based on the extraction and characterization of water soluble fractions and very few attempts have been made to evaluate the allergenic potency of water insoluble pollen constituents.

Cypress pollens are particularly liable to desiccation during their transport, facilitating the exudation and accessibility of both hydrophobic and hydrophilic intra-pollinic materials to the environment. In order to bring to light the potential role of most insoluble fractions in the cypress pollen allergy, we choose a proteomic approach based on the extraction and solubilisation of both hydrophilic and hydrophobic proteins by using some chaotropes and detergents.

Kinetic of allergen liberation in cypress pollen grains



In the usual case, the passage through the pollen exine encounters a high and virtually constant resistance. However in cypress pollen, the fragility of the exine facilitates the exudation and accessibility of hydrophobic intrapollinic materials to the environment.

Efficiency of the protein extraction

extraction methods	µg of protein per gr of pollen
H20	91
PBS	150.5
SDS	1496
*TUC	1975

Ratio: 1.7 / 10 / 17 / 21

*2 M Thourea, 7 M Urea, 2% CHAPS

1 1-D (SDS-PAGE) immunoblotting using SDS-extracts: Differential IgE binding patterns in *C. sempervirens* pollen allergy

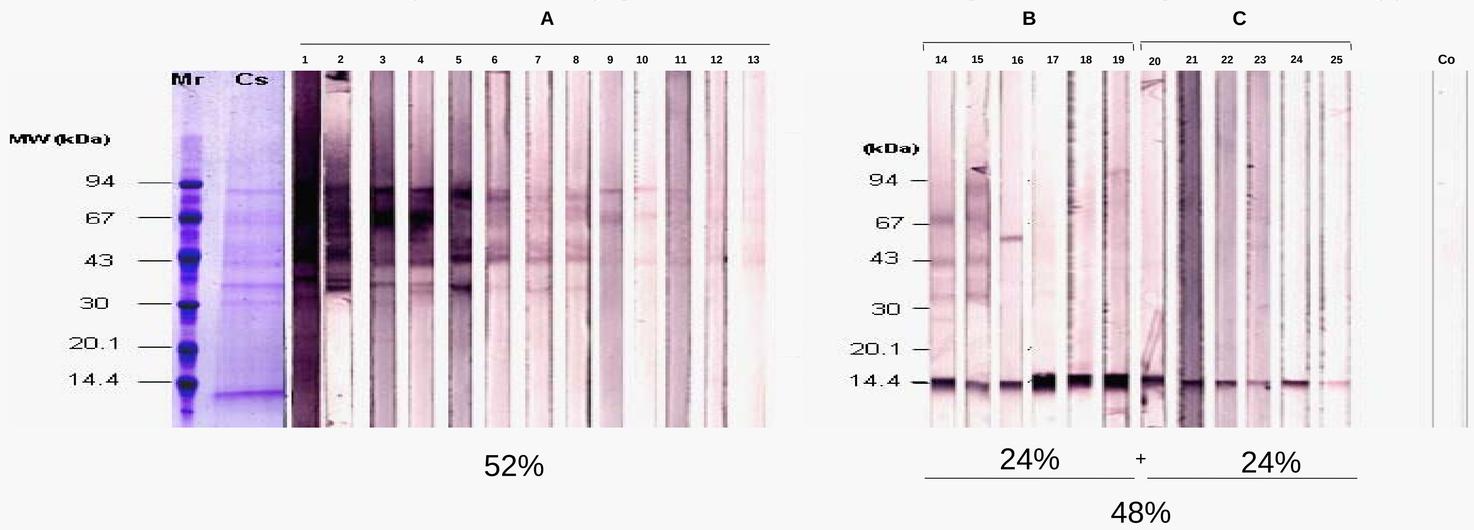


Figure1. IgE reactivity to *C. sempervirens* SDS-pollen extract. Mr represents the marker; Cs, *C. sempervirens* pollen extract; lanes 1-24, screening of 24 patient's sera by Western blotting for specific IgE antibodies to *C. sempervirens SDS*- pollen extracts and Co, the negative control.

Results

2 2-DE immunoblotting: SDS-extracts

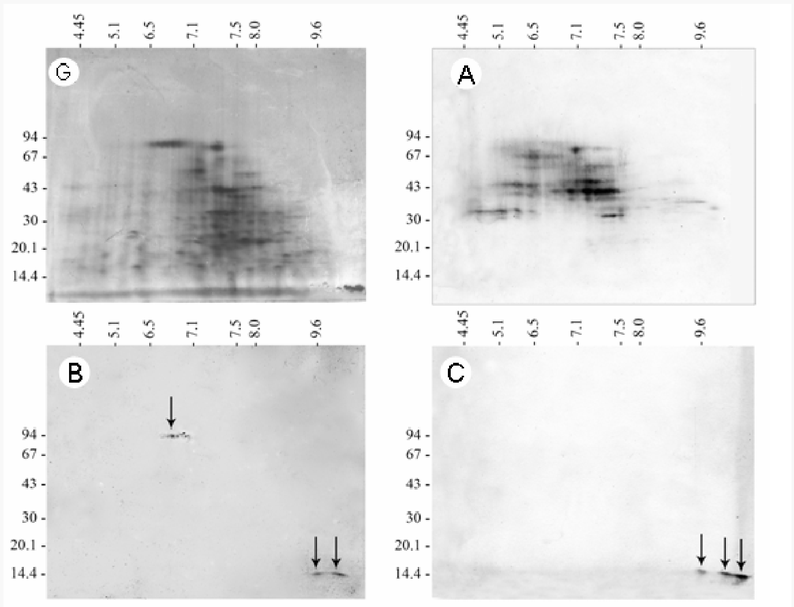


Figure 2. Silver staining of 2-DE separation (G) and typical 2-DE IgE-binding spectra of *C. sempervirens* pollen proteins (A, B, C). Molecular masses are expressed in kilo-Daltons (kDa); pI corresponds to isoelectric points. Representative IgE binding patterns A, B and C correspond respectively to 2-DE immunoblotting allergen profiles A, B and C shown in the Fig.1.

3 2-DE immunoblotting: H2O and PBS extracts

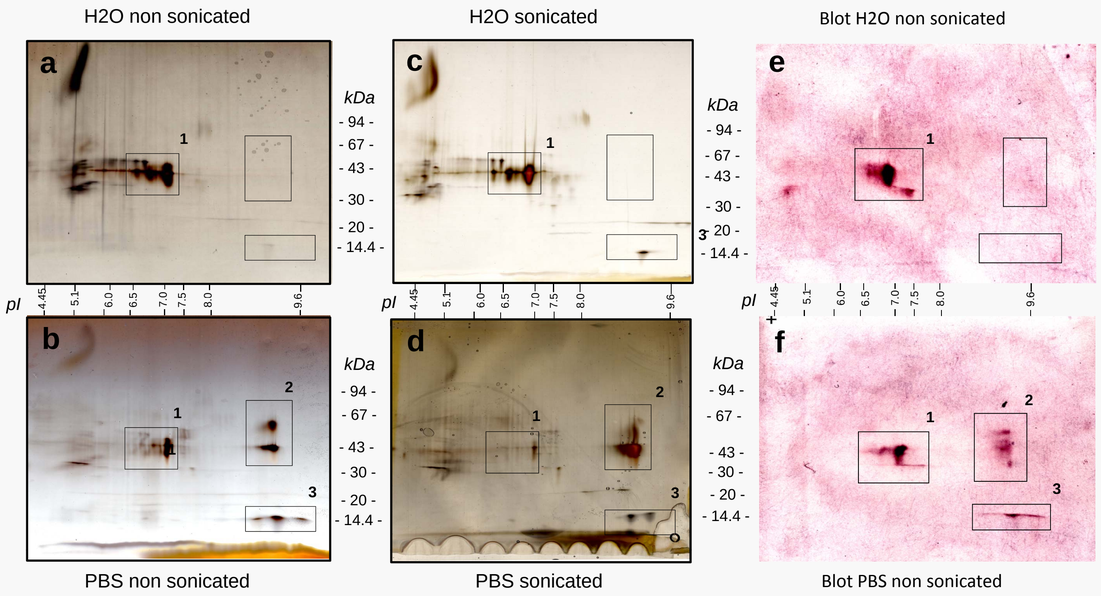
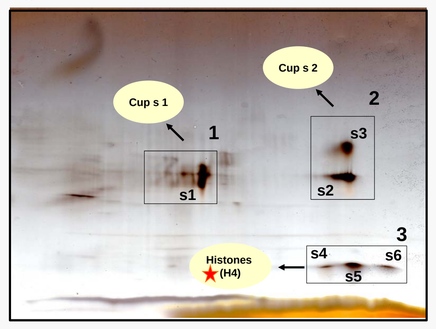


Figure 3. Comparative 2D-PAGE using H_2O (a, c) and PBS (b, d) extracts. Immunoprints (e, f) were performed with a same intermediate serum reacting to both 14 kDa allergen and high molecular weight proteins. This picture clearly demonstrates that different extraction methods result to different 2-DE allergen profiles. Aqueous extraction was very selective and essentially gave access to several isoforms of a 43 kDa protein (frame 1), while the PBS allowed the extraction of some additional basic allergenic spots of 43, 60 kDa (frame 2) as well as three basic isoforms of about 14 kDa.

MS and MS/MS peptide sequencing

Protein spots were excised and in-gel digested. Mass spectrometry analysis was performed using both MALDI-TOF-TOF and LC-ESI FT-ICR



MS/MS results

Frame	Spot (s)	Protein Name	Accession No.	Peptide count	Mascot score
1	1	Cup s 1 pollen allergen precursor (pectate lyase family)	gi|8101715	17	551
2	2	*Putative allergen Cup a 2 variant 1 [Hesperocyparis]-Polygalacturonase	gi|11819795	8	120
2	3	*Polygalacturonase OS=*Juniperus ashei* - GN=JNA2	PGLR2_JUNAS	8	133
3	4	★ Histone H4	B6TJ90_MAIZE	6	308,21
3	5	★ Histone H4	B6TJ90_MAIZE	8	437,43
3	6	★ Histone H4	B6TJ90_MAIZE	4	210,31

★Candidate, has yet to be confirmed

* A major allergen presents in Cupressaceae pollen grains, isolated and reported for the first time in *C. sempervirens*