Identification of a basic Polygalacturonase as a major *Cupressus sempervirens* pollen allergen

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INTRODUCTION

In Mediterranean countries, the common cypress currently constitutes the major source of winter/early spring respiratory allergy reaching up to 40% of the annual pollen spectrum in some cities surrounding the Mediterranean basin. Although polygalacturonases (PGs) represent major allergens in several Cupressaceae pollen grains, no protein from this family has been reported and identified in cypress (*Cupressus*) species pollen so far.

OBJECTIVE

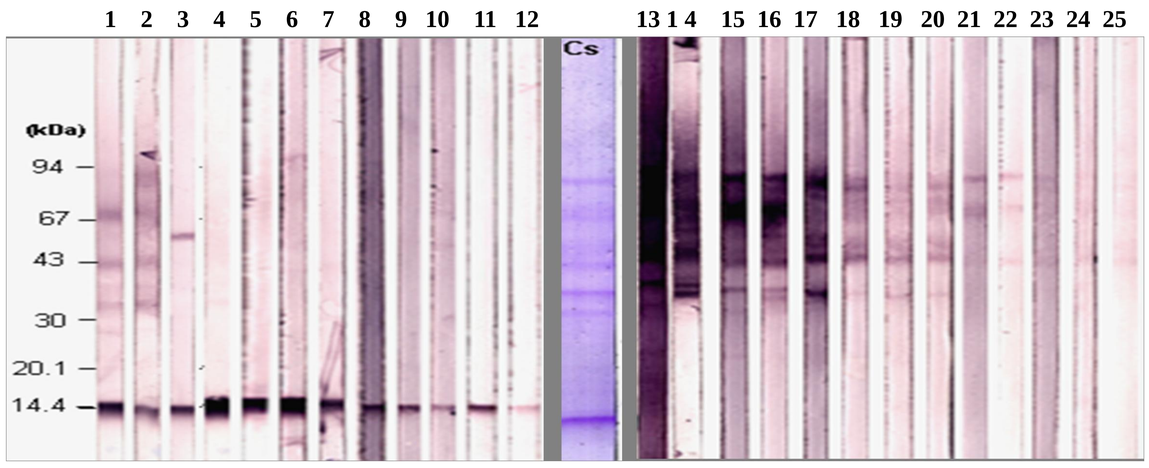
In the present study, aimed at identifying the main allergens implicated in the cypress pollen allergy using an immuno-proteomic approach, we investigate the presence of an allergen homologous to already characterized Cupressaceae PGs in common cypress (*Cupressus sempervirens*) pollen.

METHODS

The present survey describes a new procedure based on double one-dimensional gel electrophoresis (D1-DE) for the identification and characterization of allergenic components present in a complex pollen extract. By converting a protein spot previously visualized by 2-DE to an extended protein band, D1-DE circumvents the very low-abundance of some allergenic proteins detected by 2-D immunoblots. We used this technique for a multiplexed IgE immunoblotting using sera from 30 cypress pollen allergic patients. Moreover, the whole D1-DE protein band can then be excised, digested and processed for MS/MS or microsequencing analysis making protein identification more convenient.

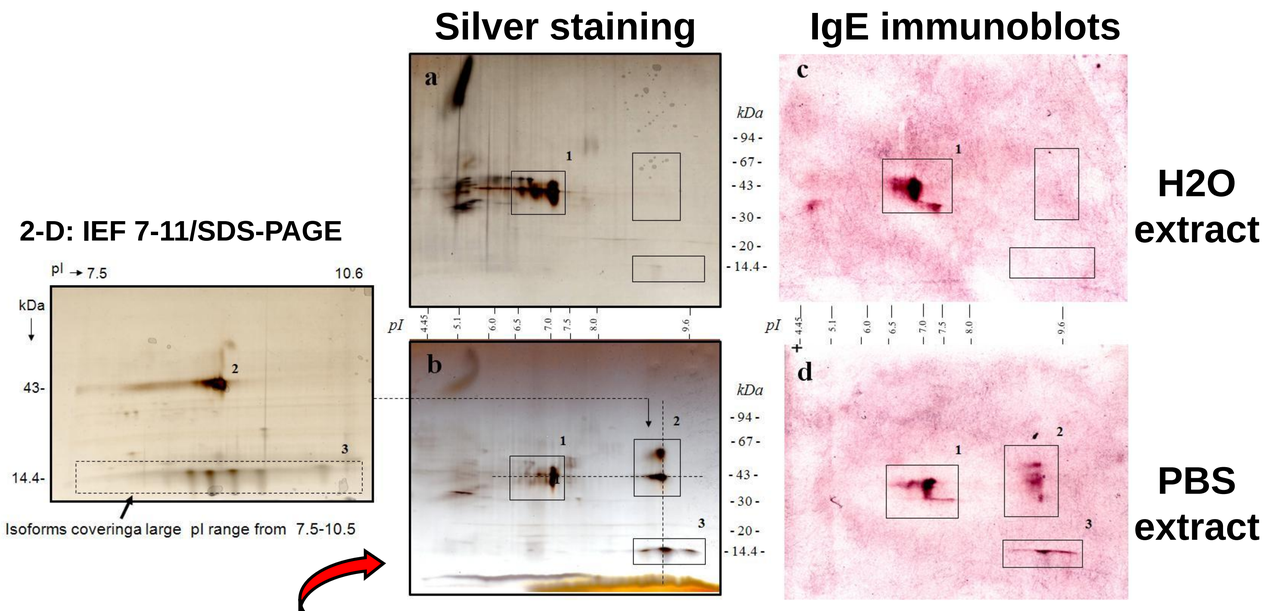
Two differential IgE reactivity patterns

1-D immunoblots (SDS-PAGE) –SDS extracts from cypress pollen



anti-14 kDa sera — anti-HMW sera

PBS extracts contain novel basic allergens (14, 43 and 60 kDa)



MS/MS identification

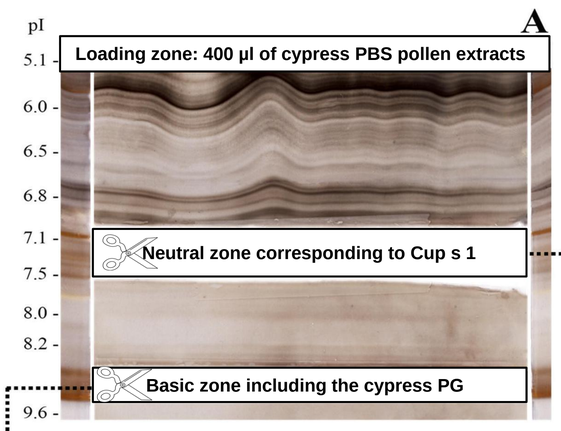
Spot no.	Protein similarity	pI	kDa	Peptide Matches	% of coverage
1	Pectate lyase Cup s 1	7	43-45	14	50
2	Polygalacturonase Jun a 2	9	43	15	48
3	REF-Hev b 1	5	14.7	6	52

Prevalence of IgE sensitization to the cypress PG: 70% (21/30)

Prevalence of IgE reactivity to Cup s 1 (a pectate lyase): 73% (22/30)

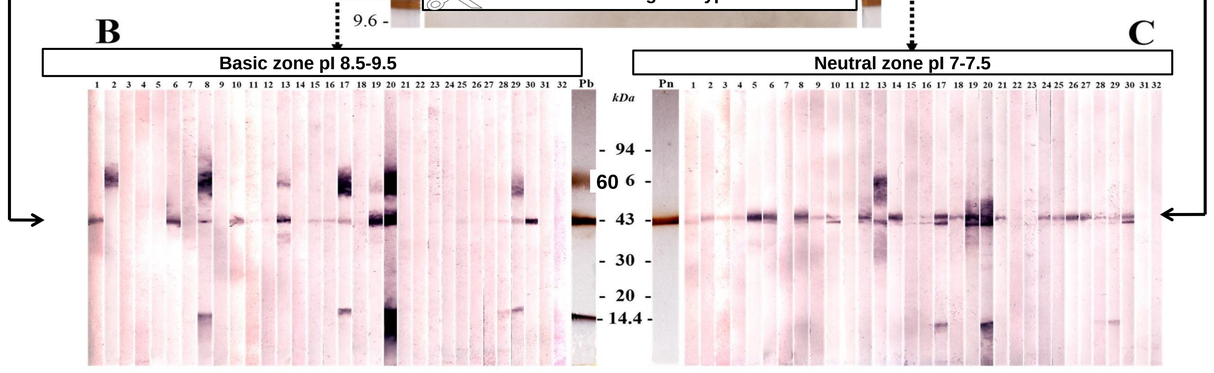
TECHNIQUE: D1-DE

IEF followed by a co-axial SDS-PAGE



IEF

Polygalacturonase — Pectate lyase Cup s 1

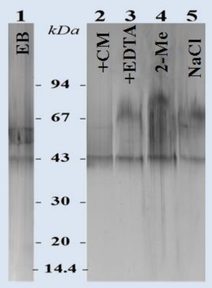


D1-DE immunoblots: IgE sreening using sera from 30 cypress allergic patients

Allergenic multiprotein complex hypothesis

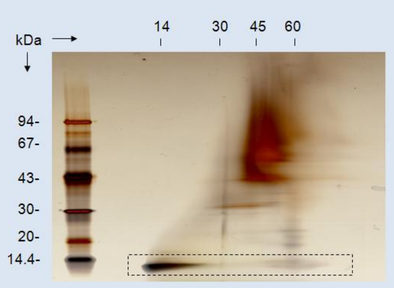
Triple 1-DE

T1-DE separation of the 60 kDa protein band

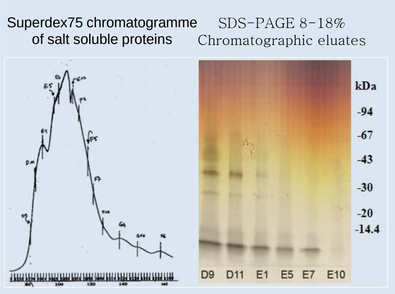


EB: Equilibration buffer
CM: Carbohydrate Mixture

2-D: BN-PAGE/SDS-PAGE

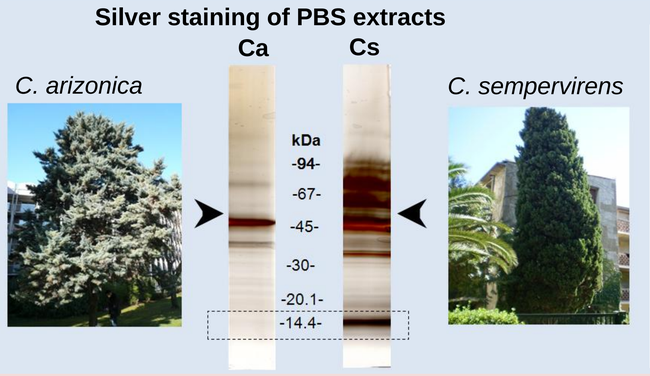


Size-exclusion chromatography

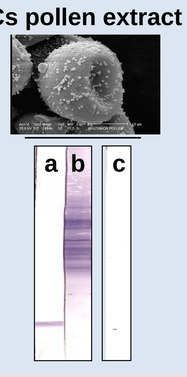


Cross-reactivity between Italian cypress pollen and latex

Silver staining of PBS extracts



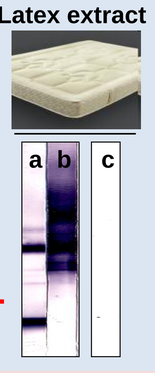
Cs pollen extract



Serum a	+ 14 kDa
Serum b	+HMW
Serum c	- control

Hev b 1 ←

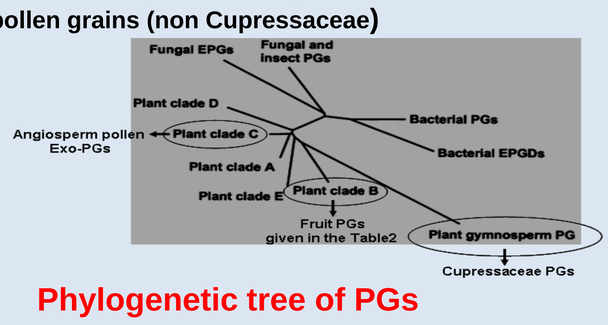
Latex extract



Pollen-food allergy syndroms (e. g. cypress pollen/peach OAS)

Amino acid sequences of cypress-cedar pollen PGs show higher homologies with members of fruit PGs than with those of other pollen grains (non Cupressaceae)

PG Clade	Common name	Sequence identity with Jun a 2 (%)
C (Pollen exo-PG)	London plane / Olive / Timothy grass	33 – 36
B (Fruit exo-PG)	Tomato / Rice / Sorghum / Kiwi / Sweet / Orange / Rape / Peach / Maize / Soybean / Muskmelon	40 – 43
B (Fruit endo-PG)	Cucumber / Grape	45 – 47
Gymnosperms (Pollen)	Arizona cypress / Japanese cypress / Japanese cedar pollen	70 - 97

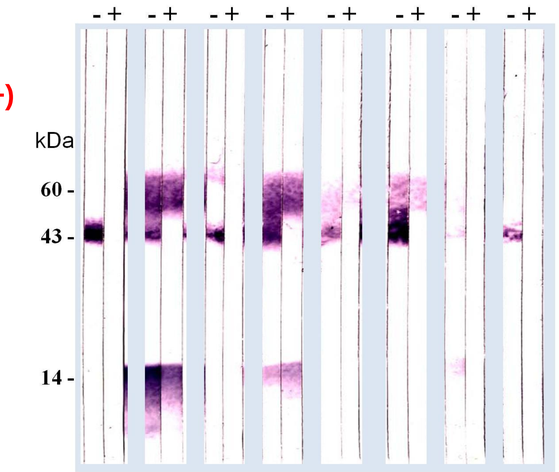


Phylogenetic tree of PGs

Cross-reactive IgE are directed against carbohydrate determinants (CCD) of the 43 kDa-cypress PG

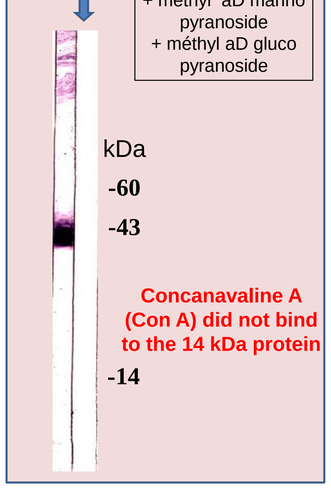
Sera incubated with Bromelain (5U/ml)

D1-DE IgE immunoblots with (+) and without (-) bromelain



The IgE reactivity to the 14 kDa allergen remains unchanged

Blot + Con A



Concanavaline A (Con A) did not bind to the 14 kDa protein

CONCLUSIONS

The present study shows that cypress pollen PBS extracts include not only Cup s 1 but also basic allergens homologous to Cupressaceae PGs and rubber elongation factor. IgE-binding to the 43 kDa PG involves bromelain-type glycan epitopes what is not the case for other basic allergens of 14 and 60 kDa. The identification and characterization of these components open new perspectives in the diagnosis and therapy of the cypress pollen allergy.