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ARTICLE

Identification of IgE binding fractions of *Oreodoxa regia* pollen antigen.

[1]Sushama Raj, R. V. and R. Prakashkumar[2]

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Abstract

Aerobiological studies conducted in Kerala (India), revealed the incidence of pollen grains of *Oreodoxa regia* as a common airborne pollen grain. Present study is concerned with the clinical, biochemical and immunological potentialities of this airborne pollen among 1500 patients with respiratory complaints. Results proved the potentiality of *Oreodoxa* regia pollen as a potent aeroallergen. Immunoblotting experiments done with serum samples gave positive results in dot blotting which confirmed the significant positivities. A group of five major allergic fractions with molecular weight ranging from 140, 110, 70, 51 and 27KD were also identified. .

Keywords: Intradermal Skin Test, SDS-PAGE, Immunoblotting, *Oreodoxa regia*.

Introduction

Nasobronchial allergy and associated illness are considered to be the most ancient but relevant health diseases among human beings. Materials that could trigger allergic reactions are referred as allergens and major being airborne are commonly called "aeroallergens". Nature of aeroallergens and intensity of hypersensitivity reactions vary from place to place depending on environmental, climatic and other geographical conditions. Majority of allergic manifestations can happen due to the inhalation of reproductive spores of plants including fungi.

The State of Kerala, being situated at the southernmost tropical belt is characterized for its remarkable diversity in flora and fauna. Aerobiological studies conducted at the Department of Respiratory Medicine during late eighties and early nineties revealed an elevated concentration of pollen grains in the atmosphere of Kerala state. Clinical studies conducted later proved that about 70-80% of them are potent allergens. As the pollen grains of *Oreodoxa regia* belong to Arecaceae are dominantly airborne in Kerala, a detailed study involving clinical, biochemical and immunological aspects was done.

Materials and Methods

Pollen grains

Pollen grains of *Oreodoxa regia* were collected in bulk and sun dried. Purity of pollen grains were checked following Cour et al. (1980) and samples with >95 % purity were considered for the study.

Antigen extraction

Antigenic extracts are the concentrations of the allergen prepared following Sheldon et al. (1967). Major steps involved in the procedure were defatting, extraction in PBS, clarification, lyophylisation using tubings of 27 x 32", toxicity and sterility studies.

Allergy evaluation

A total of 1500 patients were selected for the present study from those attending the Respiratory Allergy and Immunology Clinic, Medical College, Thiruvananthapuram, due to allergic complaints. Patients were selected with definite inclusion-exclusion criteria. Those between 10-49 years and history of respiratory allergy were included whereas patients of chronic asthma for 10 years or above, those below 10 and above 49 years of age, those on daily steroids and with other complicating diseases were excluded from the study.

For evaluating allergic response, intradermal skin testing was performed on the volar aspect of the forearm by injecting 0.02 ml of the antigen and the reactions were read after 20 minutes following Chai et al. (1975). Antigenic extract of 1:500 dilution PBS was used for testing, while phosphate buffered saline (PBS) and histamine phosphate (100 µg/ml) were used as negative and positive controls.

SDS-Poly Aacrylamide Gel Electrophoresis

For SDS-PAGE, 12% gel containing 1% SDS was used in conjugation with Tris buffer of pH 8.8. A volume of 20µl of samples containing 80µg of proteins were treated with

[1]Department of Botany, H.H.M.S.P.B.N.S.S. College for Women, Neeramankara, Thiruvananthapuram, Kerala, India. (Corresponding author).
[2]Malabar Botanical Garden and Institute for Plant Sciences, Pokkunnu, Kozhikode, Kerala, India
*Corresponding Author email: sushrv@gmail.com

2- mercaptoethanol for two minutes at 100°C. Samples were then subjected to electrophoresis at 4mA/well until the tracking dye reached 1cm from the end of the gel. The gel was stained with Coomassie Brilliant Blue R250 and calibrated with Sigma marker proteins.

Serum Samples

Sera from 25 patients who gave significant reactivity during skin testing were used for characterization studies. Among them only 21 serum samples with high reactivity in dot blotting were used for further immunological investigations.

Immunoblotting

Proteins separated through SDS-PAGE were transferred to 0.22µm nitrocellulose paper adopting semi-dry method using buffer containing 39mM glycine, 48mM Tris-HCl and 20% methanol for 45 minutes in 100V and 70mA (Schleicher and Scheul, Germany). After transfer, the blots were treated with 3% BSA in PBS to block unreacted sites. Those blots containing immobilized proteins were incubated for 48 hours with diluted serum samples (primary antibody) and HRP conjugated Goat antihuman IgE (secondary antibody) for 24hrs at 4°C. The blots were then treated with substrate solution containing 4-chloro-1-napthol and 30% H_2O_2 and continued until protein bands became suitably dark.

MALDI – TOF – MS analysis

After identification, the allergic fractions were purified and isolated using a RP–HPLC system and the molecular weight of these fractions were determined by Matrix–Assisted Laser Desorption / Ionization–Tandem Mass Spectrometry (MALDI–TOF-MS) analysis (KARTOS Analytical, Manchester, UK) using 10mg of 3, 5-dimethoxy-4-hydroxyl cinnamic acid [Sinapinic acid], 0.1% [v/v] TFA and 30% [v/v] acetonitrite as matrix. The sample was ionized by irradiation with a pulsed nitrogen laser (emission wavelength 337nm; laser power density about 106 watts/cm2) and positive ions were accelerated and detected in laser mode. Each spectrum was of the accumulation of ~50 laser shots.

Results and Discussion

Clinical Analysis

Among 1500 patients who were subjected to intradermal skin testing, 63.53% (n = 956) reacted positively to *Oreodoxa regia* pollen antigen of which 734 patients (48.93%)

Table 1. Incidence of skin test reactivity to *Oreodoxa regia* pollen antigen

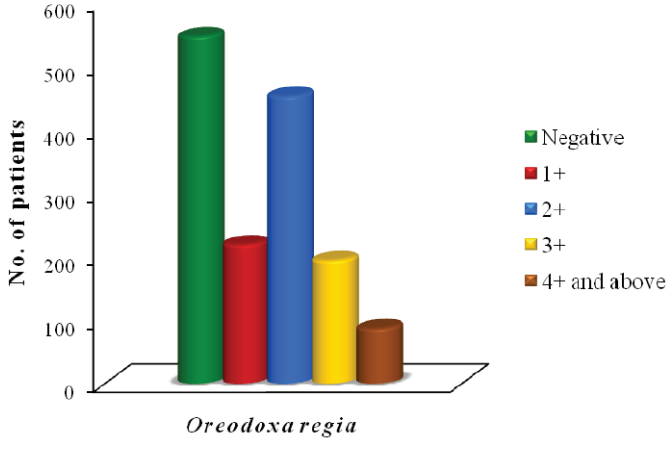
Reactivity	Highlands		Midlands		Coastalbelt	
	N	%	N	%	N	%
1+	107	21.4	86	17.2	26	5.2
2+	156	31.2	173	34.9	124	24.8
3+	-	-	78	15.6	116	23.2
4+	-	-	19	3.8	68	13.6
Total Positivity	263	52.6	356	71.2	334	66.8
Total Significant Positivity	156	31.2	270	54.0	308	61.6

Table 2. Total protein concentration (mg/gm)

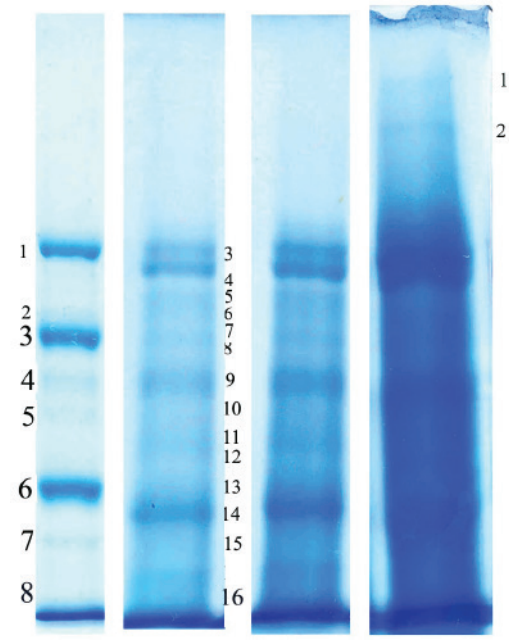
Locality	Total Protein concentration	
	Raw pollen grains	Antigenic extract
Highlands	151.91	94.34
Midlands	168.54	92.05
Coastalbelt	154.85	98.25

Table 3. SDS-PAGE profile of *Oreodoxa regia* Antigen

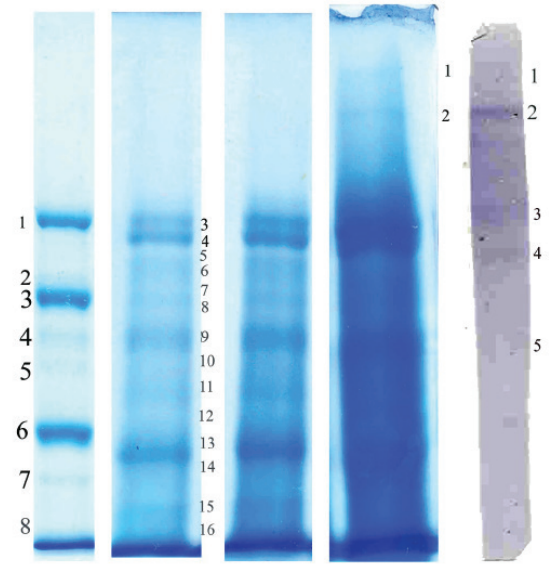
Sl.No	Molecular weight Marker	Oreodoxa regia Antigen
1	66 KD	140 KD
2	45 KD	110 KD
3	35 KD	70 KD
4	27 KD	61 KD
5	20 KD	51 KD
6	14.4 KD	48 KD
7	9.5 KD	40 KD
8	6.5 KD	35 KD
9		27 KD
10		24 KD
11		19 KD
12		16 KD
13		13.5 KD
14		12 KD
15		08 KD



Figue 1. Incidence of Intradermal Skin test reactions to *Oreodoxa regia* antigen



Figue 2. The SDS Gel developed using *O. regia* antigen showed 16 different protein bands. Line 1 represents molecular weight markers



Figue 3. Immunoblot developed using *Oreodoxa regia* antigen showed 5 different protein bands (Line 5)

reacted significantly. When individual reactions are taken into consideration, highest positivity (2+) was shown by 453 patients (30.2%) whereas 194 (12.93) and 87 (5.8%) patients responded with 3+ and 4+ reactivity respectively (Table: 01).

Aerobiological survey previously conducted in the state of Kerala (Prakashkumar et al., 1993) showed a higher incidence of the pollen grains of *Oreodoxa regia* in the atmosphere of coastalbelt. Though the protein content of the antigenic extracts showed an almost uniform level irrespective of the locality, antigens prepared with the pollen grains of coastal belt showed a higher value.

Since allergens are mainly proteins, the possibility of proteins in eliciting allergic reactions is already demon-

Table 4. Allergic fractions identified from *Oreodoxa regia* antigen

Sl. No	Allergic fractions	
	Before MALDI TOF – MS analysis	After MALDI TOF – MS analysis
01	140 KD	138.870 KD
02	110 KD	111.081 KD
03	70 KD	71.214 KD
04	51 KD	52.692 KD
05	27 KD	27.912 KD

strated. Therefore the composition of proteins and its possible role in correlating with allergic potentiality was also tried. When locality wise reactivity was analyzed, it is observed that patients from the coastalbelt showed highest significant reactivity of 61.6% to *Oreodoxa regia* antigen (Table 01). So it can be assumed that the locality of allergic patients and the protein composition of the pollen allergens of the particular locality could play a major role in eliciting allergic manifestations.

SDS-PAGE

Pollen antigen of *Oreodoxa regia* showed 16 different protein fractions ranging from 140KD to 6.5KD, which is relatively a higher index (Table: 03). Similar results were reported by Olive et al (1995), Suphioglu et al (1993) and Nilsen et al (1991) that the allergenic pollen grains contain more protein fractions than the non allergic grains. So it could be assumed that the higher number of protein fractions among *Oreodoxa regia* pollen extract could be due to its higher potentiality to elicit allergic reactions.

Immunoblotting

Dot blot assay was carried out among those significantly positive patients as a confirmatory evidence for true positive allergic response. Serum samples were collected from those 25 patients who were significantly reactive to Oreodoxa regia. Among them, 21 (84%) gave significant coloration in dot blot assay. Among these 21 serum samples, 18(86%) showed positive allergic bands in immunoblot. After immunoblotting with reactive serum, the immunoblot showed five prominent protein bands of molecular weights of 140, 110, 70, 51 and 27KD (Table: 04). Among the control serum samples of non-allergic groups, none gave significant coloration during immunoblot assay. Later molecular weights of the identified allergic fractions were confirmed through MALDI-TOF assays as 138.870KD, 111.081KD, 71.214KD, 52.692KD and 27.912KD (Table : 04).

Conclusion

Pollen grains of *Oreodoxa regia* are detected in the atmosphere of Kerala State with a dominant representation in the coastalbelt. Clinical studies proved its allergic potentiality with an allergic protein composition ranging from 140KD to 6.5KD. Immunoblotting studies have proved the presence of five allergic fractions having molecular weights of 140, 110, 70, 51 and 27KD. Identification of these specific allergic bands could help researches in developing suitable antibodies in defining proper treatment schedules.

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