

Analysis of the reactions of type I hypersensitivity to the Basidiomycetes in a Costa Rican allergic population during 2009

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Abstract

Aim: Costa Rica is a tropical country in which a high concentration of aerial basidiospores has been found, which measure between 4-13 µm and are capable of arriving to the lower respiratory system, surpassing the nasal barrier. This study aims to find possible sources of environmental contamination by basidiomycetes and to determine if it is necessary to routinely analyze this allergen in the Costa Rican population that suffer from rhino bronchial allergy.

Methods: The percentage of patients allergic to the extracts of basidiomycetes, specifically smuts like *Sporisoriumcruentum* and *Ustilagocynodontis* and rusts Pucciniagraminis subsp. graminis was determined. Also, we collected panicles of *Panicum maximum* to assess their contamination by basidiomycetes.

Results: We found that 59.23% of patients were allergic to mushrooms and that the fungi type for which more patients had allergy is the basidiomycetes. At the same time, a 100% of the collected panicles were contaminated with a fungus of the genus *Ustilago*.

Conclusion: Most of the patients studied were allergic to basidiomycetes, therefore, we strongly recommend that Costa Rican allergists constantly test for these extracts.

Keywords: Basidiomycetes, allergy, fungi, *Ustilago*.

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In our country there have been several studies on the identification of the different species of basidiomycetes, based on the search for basidiocarps producers, in areas like Monteverde and Volcano Poás.¹⁻³ However, in the Central Valley it is not usually observed a large concentration of basidiocarps that justifies the high rate of basidiospores (23% of total air fungal spores) (Jaikel, et al., thesis to opt for a Bachelor's degree in Microbiology and Clinical Chemistry at UCR) in the environment which suggests that the major basidiomycetes in this area are the rust type fungi or the coal type fungi, which parasitize various plants, but do not produce basidiocarp.⁵⁻⁸ An example of this is found in grasses such as *Panicum maximum* (Guinea), which is found in large areas of the country, both in the Central Valley and beyond,⁹ and shows that its panicle is colonized by a fungus of pigmented spores.

In 1951, there were the first reports of the relationship between basidiospores and respiratory symptoms,¹⁰ as they are known as contact allergens and inhalation allergens causing bronchial asthma, chronic cough, hypersensitivity pneumonitis, contact dermatitis¹¹⁻¹³ and even anaphylaxis.¹⁵ But, not only are spores cause allergy: studies with the *Pleurotus ostreatus* and the *Coprinus quadrididus* showed that extracts from the pile and stipe contain allergens in approximately equal amounts or in excess of the amounts present in extracts of spores.¹⁰

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The use of farms within buildings, specialized for growing edible mushrooms, has become popular in Japan, North America and Europe, due to its great success. Unfortunately, during harvest, millions of spores are released into the environment and they cause the “fog of spores”, which can obstruct the visibility in these rooms. This is the main reason that in a study of workers who grow *Hypsizigus marmoreus* (Bunashimeji) one third presented chronic cough, after a few months of starting to work, and three years later, up to 90% of the workers showed positive precipitins in their serum to fungal spores, 40% left the farm due to severe respiratory allergic disease and 5% developed hypersensitivity pneumonitis.¹⁵

Regarding the immune response, macrophages / monocytes, NK cells and NK T cells are crucial in the innate immune response mounted against these pathogens and host T lymphocytes are important during the adaptive response. Because the spores of Basidiomycetes are composed of protein and lipid complexes it is believed that CD1 is involved in their recognition. The CD1b is an important marker of monocytes and activated dendritic cells and it also presents glycolipids from the outer cell wall of *Mycobacterium tuberculosis* to the non conventional T cells, the double negative $\alpha\beta$ T and $\gamma\delta$ T. Furthermore, the CD1d recognizes and binds to internal and external glycolipids stimulating NK cells. Finally, it has been shown that in these patients there is an increase in IL-13, a decrease in IFN- γ , an increase of Th2 lymphocytes and Th2/Th1 ratio.¹⁵

IL-13 plays an important role in the development of airway hyperresponsiveness and in isotype switching to IgE production by B lymphocytes, like IL-4. Furthermore, it induces an independent asthmatic phenotype of lymphocytes, suggesting that it acts, at least in mice, directly on non-immune cells in the bronchial tissues. This allows the inference that asthma in workers of mushroom farms, can be caused by inflammation mediated by Th2-type cytokines, especially IL-13.¹⁵ Basidiomycetes are capable of inducing, increased IL-13, stimulation of the cascade of allergic inflammation and low levels of IFN- γ , which by itself is poor in atopic population, by a defect not well understood until now.¹⁶⁻¹⁸

Despite the importance of basidiomycetes in public health, there are few or almost no health centers, both in Costa Rica and the rest of Central America, which have incorporated extracts from basidiomycetes in their kits for the diagnosis of immediate hypersensitivity diseases, and only Zygomycetes/Ascomycetes are tested. Thanks to the above, this study aims to analyze the type I hypersensitivity reactions to basidiomycetes in an allergic population in Costa Rica, from January to December 2009.

The first aerobiological study conducted in Costa Rica, demonstrated the importance of basidiomycetes in air samples from schools in the province of Heredia, and it was made in 2008 (Jaikel, et al., Thesis to qualify for the title of

degree in Microbiology and Clinical Chemistry at UCR), and it warned about the lack of national or central-american research on such allergens.

This is the first study conducted in the country, which aims to demonstrate the potential sources of pollution responsible for high concentration of aerial basidiospores and pretends to assess routine analysis with extracts from different basidiomycetes in the evaluation of the allergic patient.

Materials and methods

Study population: We conducted a retrospective study based on the records of the Allergy Section in the Heredian Medical Clinic, between January and December 2009. We studied 130 atopic patients from different regions of the country: 81 female and 49 male, with an age range of 6-86 years (mean 35.5 years).

First, we determined the percentage of patients that were allergic to extracts of basidiomycetes: coal type *Sporisorium cruentum* and rust type *Puccinia graminis* subsp. *graminis*, as a whole, by linking data from positive prick tests for these fungi, in 2009, with the total population of patients of the Heredian Medical Clinic during that same year. Then, the percentage of patients allergic to rust type and type coal fungi was obtained separately in order to assess which is the most important.

As part of the Clinics protocol, four mixtures are used for testing fungi Ascomycetes, Zygomycetes and imperfect fungi, namely:

- a. Blend A or mixture of *Aspergillus* spp.: *Aspergillus clavatus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus* spp.
- b. Blend B or mixture of hyaline fungi other than *Aspergillus*: *Chaetomium* sp. *Fusarium* sp. *Geotrichum* sp. *Mucor* sp. *Paecilomyces* sp. *Penicillium* sp. *Rhizopus* sp. and *Trichophyton rubrum*.
- c. Blend C or mixture of sooty fungus: *Alternaria* sp. *Cladophiala* sp. (*Cladosporium*) *carrioni*, *Cladosporium cladosporioides*, *Cladosporium* sp. and *Curvularia* sp.
- d. Blend D or mixture of yeast: *Candida albicans* and *Rhodotulalarubra*.

Data were collected concerning the percentage of patients allergic to each of these fungal mixtures and the associated clinical manifestations of such patients. Data on sex, age and place of residence, were analyzed for possible

demographic relationships regarding patients allergic to Basidiomycetes. Then, we determined the percentages of each disease in the total of allergic patients to these extracts. However, because the Heredian Medical Clinic patients are, mostly, atopic patients, the information obtained on the various manifestations was analyzed comprehensively with main environmental allergens: dust mites (*Dermatophagoides pteronyssinus*, *Tyrophagus putrescentiae* and *B. tropicalis*) and grass pollens, as in Costa Rica polysensitization is the rule, not the exception, in the allergic population.

We used the chi-square test to determine the association of variables between different types of fungi and the clinical manifestations.

The Bioethics Committee of the University of Costa Rica approved the research protocol (Session No. 206, VI-Doc 8897-2010), as it was within the ethical and humane principles of research. The Center for Diagnosis and Treatment of Allergy approved the revision of the clinical records of these tests.

Collection of panicle *Panicum maximum* contaminated with Basidiomycetes: we conducted a field study to find basidiomycetes pollution in the province of Heredia, Costa Rica.

The plant chosen was the grass *Panicum maximum*, which is distributed as wild foliage at the edge of the road, up to 1100 meters above sea level, especially between May

and November.¹⁹ The collection took place in the following cantons: Central Canton (Mercedes district), Barva and San Pablo. The plants were cutted at the level of stem and they were placed in individual plastic bags and they were labeled. There were a total of 23 panicles, which were analyzed under the light stereoscope. The basidiomycetes spores that were found underwent a wet montage with blue lactophenol, which helped with the identification of the genre it belonged.

The contaminated pollens were sown in glass bottles with 25 ml of dextrose agar Sabouraud, and stored at room temperature for one month.

Basidiomycetes extract preparation isolated from *Panicum maximum* for skin testing: The material obtained from the culture in Dextrose Agar Sabouraud was washed three times with acetone to remove the lipids, and then fungal structures were preserved in liquid nitrogen.

Allergenic material was extracted in phenolated saline buffer 0.4% (Evans solution) with continued agitation for 48 hours at 4 ° C at a concentration weight / volume (W / V) of 1/10. It was centrifuged at 3500 rpm for 30 minutes. The supernatant was removed and filtered through a sterile milliporomembrane of 0.22 micrometers.

Standardization of extract for skin testing: an in vitro standardization was performed, in which the amount of total proteins was specified by the Dumas method, for the crude extract obtained from the basidiomycete isolate

Table 1: Percentaje of allergic patients to fungi, seen in HMC January and December, 2009

Type of Fungus	Number of allergic patients	% of allergic patients X from the total	% of allergic patients from the total al allergic patients to basidiomycetes
Any fungi*	77	59,23	Not applicable
<i>Aspergillus</i> spp.	17	13,08	Not applicable
Hyaline fungus different from <i>Aspergillus</i> spp.	35	26,92	Not applicable
Fuliginosus fungus	17	13,08	Not applicable
Yeast	40	30,77	Not applicable
Basidiomycetes in general	53	40,77	100,00
Basidiomycetes coal type**	27	20,80	50,94
<i>Basidiomycetes coal type: Ustilagocynodontis</i>	21	16,15	39,62
Basidiomycetes coal type <i>Sporisorium cruentum</i>	19	14,62	35,85
Basidiomycetes rust type: <i>Puccinia graminis subsp. graminis</i>	42	32,31	79,25
Only basidiomycetes	25	19,23	47,17
Fungus different from basidiomycetes	24	18,46	No aplica

*Ascomycota, Basidiomycota, Zygomycetes y imperfect fungus
 **Any combination of coal type fungus

Table 2: Distribution according to sex and positivity, to the prick test of the allergic patients to basidiomycetes, in the allergic population seen in HMC, January, December, 2009

Patients	Amount	% of positivity
General total*	130	40,77
Males	49	40,82
Females	81	40,74

* Any diagnostic combination

Table 3: Relationship between the age and prick test positivity for basidiomycetes, in patients seen in the HMC, January, December, 2009

Age (years)	Number of patients	% of positivity
0-12	11	45,45
13-40	72	45,83
> 40	47	31,91
Total	130	40,77

Table 4: Distribution by province, of the allergic patients to basidiomycetes, seen in the HMC January, December, 2009

Province	Number of patients
Alajuela	13
Cartago	11
Guanacaste	6
Heredia	30
Limón	1
Puntarenas	5
San José	64

panicle of *Panicum maximum* and for the three extracts of commercial basidiomycetes GREER (*Sporisorium cruentum*, *Ustilagocynodontis* and *Pucciniagraminis* subsp. *graminis*). All four extracts are at a concentration of 1/10 P / V. Based on the results, we determined the concentration at which the extract can be used.

Results

As seen in Table 1, from the patients studied, 59.23% are allergic to fungi, being basidiomycetes 40.77%, yeasts 30.77%, hyaline fungi other than *Aspergillus* spp. 26.92%,

Table 5: Distribution of allergic patients to basidiomycetes, seen in the MHC, by diagnosis January, December, 2009

Diagnosis	Number of patientes	% of positivity
Only rhinitis (R)	2	3,77
Only conjunctivitis (C)	1	1,89
R+C	21	39,62
R+A*	1	1,89
R+D**	4	7,55
R+A+C	20	37,74
R+C+D	2	3,77
R+A+C+D	2	3,77

*A, bronchial asthma
**D, atopic dermatitis

and *Aspergillus* spp. and sooty fungus, both 13.08%. Similarly, in the most important group of fungi, which is the Basidiomycetes, 79.25% corresponds to the allergy against rust fungus *Pucciniagraminis* subsp. *graminis*, and 50.94% against the coal type.

According to the analysis of demographic data (Tables 2, 3 and 4), it should be noted that there are no significant differences found between the percentage of female patients allergic to Basidiomycetes as compared to males, or between age groups, but there was a decrease of positive prick tests in the population over 40 years, with respect to the one under that age. It was not possible to perform the analysis by province, as the number of patients was not sufficient to assign them any statistical value.

Table 5 shows the distribution of these allergic patients by diagnosis. The main clinical features associated with patients Heredian Medical Clinic, that were allergic to basidiomycetes, were: rhinitis and conjunctivitis and rhinitis, conjunctivitis and asthma. We found no patient whose diagnosis was exclusively bronchial asthma or atopic dermatitis. At the same time, 88.68% of patients allergic to any basidiomycete are allergic to house dust mites and storage mites, and 81.13%, to grass pollens, as befits the rule of the polysensitization in the costarican atopic patient.

When performing the chi-square test for allergy variables *Aspergillus* spp. against a diagnosis of asthma, we found that these two variables are dependent, but not with the other types of fungi and this disease (Table 6).

Finally, 100% of panicles had fungal contamination (characterized by black spots) in at least 25 of its inflorescences (Figure 1) identifying the isolation as a basidiomycete coal type belonging to the genus *Ustilago* (Table 7).

Discussion

The results demonstrate that the Basidiomycetes corresponds to an important group as allergens, since they are causing atopic reactions in 40.77% of patients analyzed, and that 59.23% of allergic patients to fungus are allergic to them. In turn, what is most important is that 19.23% of patients are allergic to these fungi only, so it can be under diagnosed in their allergic disease, if it is not routinely tested with the Basidiomycetes allergens. In view of the results, we recommend using these fungi as part of the routine in symptomatic patients with negative skin tests. It should be emphasized that the main types of these fungi that cause allergies are the rust type, so the source of this sensitization should be determined. Furthermore, a higher allergenic potential is reported, because it has about 6 times more protein than coal type.

There was no variation in the percentage of positivity obtained to the studied allergens by sex. Similarly, there is no link between the age groups of 0-12 years and 13 to 40 years, but there is a link between them and the group older than 40 years, which may be due to lower type I reactivity in this population, as it is described that the most allergic activity occurs in children and young people. This

Table 6: Percentage of allergic patients to different fungus, seen in the HCM, by diagnosis of bronchial asthma January, December, 2009

Fungus type	Number of patients	Number of patients with bronchial asthma	Percentage (%)
<i>Aspergillus</i> spp.	17	11	64.70
Hyaline fungi different from <i>Aspergillus</i> spp	35	16	45.71
Fuliginosus fungi	17	7	41.18
Yeast	40	21	52.50
Basidiomycete	53	23	43.40

Table 7: Protein concentration determination from basidiomycetes extracts, mg/mL

Basidiomycetes extracts	Concentration (mg/mL)
<i>Ustilago</i> sp. Isolated from <i>Panicum maximum</i> 1/10 P/V	10
<i>Ustilago cynodontis</i> 1/10 P/V	10
<i>Sporisorium cruentum</i> 1/10 P/V	12
<i>Puccinia graminis</i> subsp. <i>Graminis</i> 1/10 P/V	60

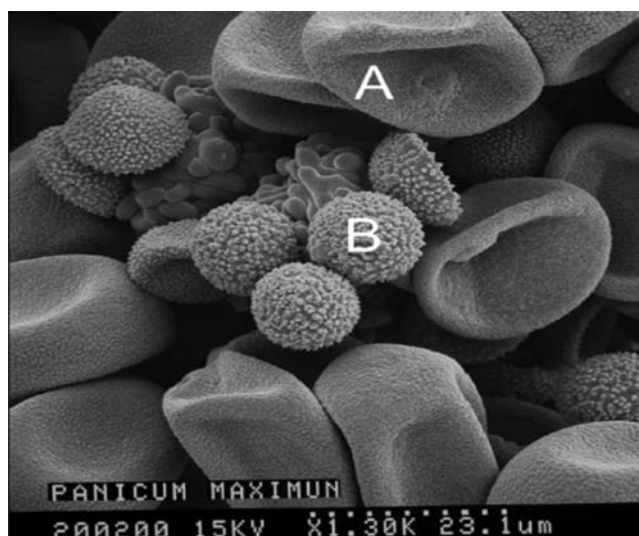


Figura 1. Electronic microscopy of grass pollen grains *Panicum maximum* (A) contaminated with *Ustilago* spores (B). *Ustilago* spores are 7-11 μ m, so they can reach lower respiratory tract.

phenomenon is also reported for newborns and infants, however, in our study population the youngest patient was 6 years.²⁰

With regard to the symptoms caused by basidiomycetes and fungi in general, literature reports that they are a cause of asthma,¹⁴ but in this study population it was not the case, since there was no relationship of dependence between variables. Association was found only with this diagnosis in patients allergic to *Aspergillus* spp., which corresponds to spores that have a size of 2 micrometers or less, so they can reach the pulmonary alveoli. Not so with the Basidiomycetes, as their spores, although small, are larger than the 4 μ m.^{11, 21}

The majority of allergic patients were diagnosed with acute basidiomycetes of allergic rhinitis and allergic conjunctivitis and allergic rhinitis, bronchial asthma and allergic conjunctivitis, but no significant association with any particular pathology. This is because the allergic population of tropical countries is a population with sensitivity to many different allergens, including allergy to house dust mites and storage mites, to grass pollen, so it is not surprising that 88.68% of allergic patients allergic to mites are also allergic to basidiomycetes, and 81.13% also allergic to pollens.

The *Panicum maximum* panicles harvested were entirely colonized by the fungus *Ustilago* sp. identified as coal type, showing that these plants are an important source of fungal perennial allergens in the environment, and when they disperse at air level,⁷ they easily come in contact with the allergic patient. As mentioned above, almost 80% of allergic patients are allergic to basidiomycetes rust type fungi, so it is proposed as a continuation of this study, trying to locate sources of fungal contamination by rust, taking as a starting point the coffee plantations, which are often colonized with coffee rust: *Hemileia vastatrix*.

As medicinal chemistry evolves, active materials has been prepared and synthesized exactly with a known composition, which can be administered in precise amounts and with confidence in the reproducibility of the effects of the different drugs used. However, the allergenic extracts are still the exception to this trend. Therefore, the purpose of standardization of allergens is to offer the allergists, extracts with known concentrations of allergens for diagnosis and immunotherapy of allergic diseases.²²

The protein concentration for the two extracts obtained from *Ustilago* sp. (commercial and homemade) are equal, which shows that nationally allergens can be produced based on indigenous raw materials, with the same quality as in the transnational laboratories. It should be noted that one of the reasons for the variability in the mold studies for type I hypersensitivity, is the amount of the own enzymes of the fungus, which cause fungal allergens to be unstable while stored, such as allergens of domestic cockroach, so it is recommended to change them often with short due dates, of about a year, in the aqueous extracts, making the diagnosis difficult and expensive. The importance of environmental analysis should be highlighted to determine the biodiversity characteristics of each region of the country and to offer each patient a more accurate diagnosis and an effective immunotherapy.

Conflict of interest: The authors report no conflict of interest.

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