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Extraction and Purification of the Potential Allergen Proteins from *Botryotinia fuckeliana*

Mustafa KOROGLU
Gaziantep University

Ibrahim Halil KILIC
Gaziantep University

Isik Didem KARAGOZ
Gaziantep University

Mehmet OZASLAN
Gaziantep University

Abstract: An allergy is a disease in which the immune system makes an inflammatory response to a harmless antigen. Any antigen that causes an allergy is called an allergen. Allergens may be inhaled or ingested, or they may come into contact with the skin. According to the data of the World Allergy Organization (WAO), the prevalence of allergies in different countries varies between 10-40%. Pollen, mold, animal hair, house dust mite, medicines, and foods are the most common allergen agents. Common mushrooms in nature have the potential to produce allergenic proteins. *Penicillium*, *Botryotinia*, *Aspergillus*, *Rhizopus*, and *Mucor* species, which are allergic fungi, are widely found in nature. In recent years, the cases of allergies caused by molds have increased significantly and studies to determine the causing allergens have accelerated. *Botryotinia fuckeliana* Pers. is a cosmopolitan necrotrophic pathogen infecting more than 200 plant species in temperate and subtropical climates, some of which are of high economic importance (e.g., grapes, strawberries, solanaceous vegetable). In Europe, maximal spore counts occur during late summer. Prevalence of skin test reactivity, i.e., immediate wheal and flare, to *B. cinerea* in Europe is comparable to that of *Aspergillus* spp. and greater than that of *Cladosporium* and *Penicillium* spp. Since the prevalence of *B. fuckeliana* spores and skin test reactivity is significant, further characterization of these allergens is warranted. *Botryotinia fuckeliana* was used in our study. *Botryotinia fuckeliana* produced in our laboratory was collected and allergen fungus protein was extracted by 2 different extraction methods. By preparing protein samples from prepared mushroom extracts, the total concentration of potential allergen proteins was determined by the BCA method. According to the data obtained, it was determined that the protein concentration of the mushroom samples dried by that were subjected to dialysis was higher than ethanol.

Keywords: Allergy, *Botryotinia fuckeliana*, Allergen protein, BCA

Introduction

Allergies develop when a person's immune system overreacts to substances that are usually harmless. The first time a person is exposed to an allergen, they do not usually experience a reaction. It often takes time for the immune system to build up a sensitivity to the substance. In time, the immune system learns to recognize and remember the allergen. As it does so, it starts making antibodies to attack it when exposure occurs. This buildup is called sensitization. Allergic diseases usually affect the skin and mucosal tissues such as sinuses, lungs, and intestines (Tao & Raz, 2015).

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According to the data of the World Allergy Organization, the prevalence of allergies in different countries varies between 10-40% (Pawankar, 2011). It is possible to encounter allergic diseases seasonally or throughout the year. Seasonal allergic reactions are caused by fungal spores, pollen, insecticides, indoor and outdoor mold fungi, house dust, and animal hairs that persist throughout the year (Şimşekli, 1994). In recent years, many researches have been carried out in the field of aeromicrobiology, which is very popular in the world, sports calendars of many cities in our country and abroad have been published, and atmospheric sports concentrations are announced and announced to the public through meteorological bulletins (Çeter & Pınar, 2008).

Pollen, fungi, and house dust mites are the most common allergens. Fungi or fungal spores that can be found in the other environment can hang in the environment for a long time due to the effect of the airflow from their location (Simon-Nobbe et al., 2008). Mushrooms, which are among the most harmful organisms for humans, are equally useful organisms due to their use in different areas such as the decay of organic substances in the ecosystem, the production of species consumed as food, and the development of biotechnology, such as the synthesis of biofuels, enzymes and drug active substances (Kendrick, 2000; Esch, 2017). Mushrooms, which have the most species after insects, have the potential to produce allergenic proteins. These organisms, which have a wide distribution area, are estimated to constitute more than 90% of the biomass in the world (Kendrick, 2000).

Airborne fungal spores occur widely and often in far greater concentrations than pollen grains. Immunoglobulin E-specific antigens (allergens) on airborne fungal spores induce type I hypersensitivity (allergic) respiratory reactions in sensitized atopic subjects, causing rhinitis and/or asthma. The prevalence of respiratory allergy to fungi is not known with certainty, but is estimated to be between 20% and 30% of atopic (allergy-prone) individuals or up to 6% of the general population.

Diagnosis and immunotherapy of allergy to fungi require well-characterized or standardized extracts that contain the relevant allergen(s) of the appropriate fungus. Production of standardized extracts is difficult since fungal extracts are complex mixtures and a variety of fungi are allergenic. *Penicillium*, *Aspergillus*, *Rhizopus*, and *Mucor* species, which are allergic fungi, are widely found in nature. In our country, it has been reported that 614 patients with respiratory tract allergies develop allergic reactions against *Aspergillus fumigatus*, *Trichophyton rubrum*, *Mucor*, *Penicillium notatum*, *Aspergillus niger*, and *Alternaria tenuis* (Güneser et al., 1994).

In connection with allergy and asthma, the typical fungal genera investigated are *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium*, probably because they are very often the most prevalent genera in ambient air. However, the diversity of species can be rather high, both within the 2–4 most prevalent genera and in less prevalent general (Lugauskas et al., 2003). For example, 100 genera containing 359 species when investigating the fungal composition indoors. Furthermore, many fungal species from at least 80 genera, have been shown to have allergenic potential. One of these fungi present in ambient air is *Botryotinia fuckeliana*.

Botryotinia fuckeliana, an airborne necrotrophic fungus that attacks more than 200 plant species, causes gray mold disease of many economically important crops, including vegetables, ornamentals, bulbs and fruits (Plesken et al., 2015a).

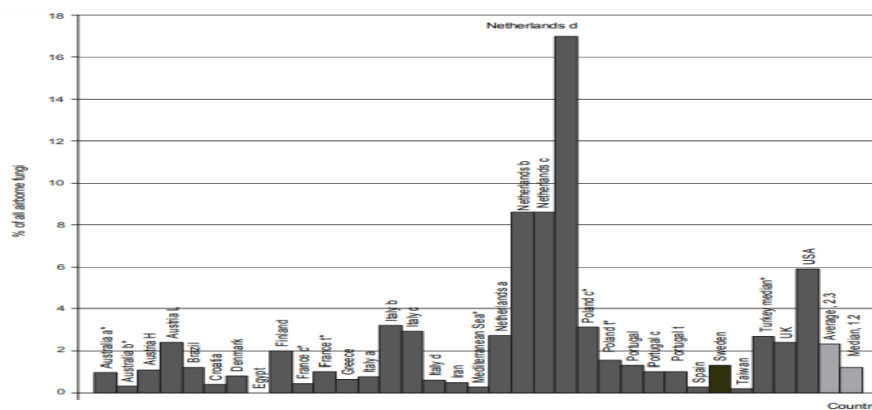


Figure1. Prevalence of outdoor *Botryotinia* in percent of all airborne fungi (black bars).

Method

Preparation of *Botryotinia fuckeliana* Extracts

The mushroom samples used in our study were purchased commercially and reproduced in our laboratory. *Botryotinia fuckeliana* spores, which were cultivated on PDA medium, were left to incubate for 7-14 days at +25°C. It was collected after morphological examination by staining with cotton blue. The resulting mushrooms were treated in chloroform-methanol and ethanol on a magnetic stirrer for 24 hours. The dried mold samples were digested in PBS and dialyzed. Protein concentrations were determined by extracting the obtained extracts into %5 SDS Buffer.

Determination of Total Protein Concentration

The total protein concentration of the mushroom extracts was made using the bisinconic acid (BCA) method proposed by Smith et al. (1985). Commercially purchased BCA Macro Assay Kit (Serva Electrophoresis GmbH) was used to determine protein concentration. BCA analysis was performed following the protocol suggested by the manufacturer (Walker, 2002).



Figure 2. BCA working diagram

Results and Discussion

In our study, proteins of *Botryotinia fuckeliana*, one of the allergenic fungi, were extracted with 2 different extraction protocols. The amount of extracted proteins was measured by the BCA method. In the protocol of the mushroom extracts prepared in the study using ethanol, sufficient protein amount could not be obtained and it was observed that it was not a suitable protocol for our study. In the protocol using chloroform-methanol, the amount of protein was determined as 0.660 mg/mL. Similar results were obtained with the amount of protein stated in previous studies in chloroform-methanol extraction (Wójcicka, 2014).

Table 1. Total protein concentration values of *Botryotinia fuckeliana* extracts measured by BCA assay

| Allergen Name | Protein Concentration (mg/mL) |
|--|-------------------------------|
| Botryotinia fuckeliana (Ethanol) | ----- |
| Botryotinia fuckeliana (chloroform-methanol) | 0,660 |

Recommendations

In recent years, allergy cases caused by molds have been increasing. For this reason, studies to determine the allergen proteins of fungi commonly found in nature have gained importance. In our study, protein concentrations were determined by preparing *Botryotinia fuckeliana* extracts, which is one of the allergen fungi and used in allergen kits. The data obtained from this study form the basis for the production of alternative domestic kits to imported kits used in the diagnosis and treatment of allergy patients with advanced studies.

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References

- Çeter, T. Y., & Pinar, N. M. (2008). *The concentration of atmospheric pollen and spores of Kastamonu province (Central Region) and effects of meteorological factors on this concentration* (January 2006-December 2007) (Doctoral dissertation, Ankara University Graduate School of Natural and Applied Sciences Department of Biology).
- Esch, R. E., & Codina, R. (2017). Fungal raw materials used to produce allergen extracts. *Annals of allergy, asthma & immunology : Official publication of the American College of Allergy, Asthma, & Immunology*, 118(4), 399–405. <https://doi.org/10.1016/j.anai.2016.05.012>
- Güneser, S., Atici, A., Köksal, F., & Yaman, A. (1994). Mold allergy in Adana, Turkey. *Allergologia et immunopathologia*, 22(2), 52–54.
- Kendrick, B. (2000). *The fifth kingdom* (pp. 227-247). Hackett Publishing
- Pawankar, R., Canonica, G. W., Holgate, S. T., Lockett, R. F., & Blaiss, M. S. (2011). *WAO white book on allergy* (pp. 102-110). Milwaukee, WI: World Allergy Organization, 3, 156-157.
- Plesken, C., Weber, R. W. S., Rupp, S., Leroy, M., & Hahn, M. (2015a). Botrytis pseudocinerea is a significant pathogen of several crop plants but susceptible to displacement by fungicide-resistant B. cinerea strains. *Appl. Environ. Microbiol.* 81, 7048–7056. doi: 10.1128/AEM.01719-15
- Lugauskas, A., Krikštaponis, A., & Šeškauskas, V. (2003). Species of conditionally pathogenic micromycetes in the air of dwellings and occupational premises. *Indoor and Built Environment*, 12(3), 167-177.
- Simon-Nobbe, B., Denk, U., Pöll, V., Rid, R., & Breitenbach, M. (2008). The spectrum of fungal allergy. *International archives of allergy and immunology*, 145(1), 58–86. <https://doi.org/10.1159/000107578>
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goetze, N. M., Olson, B. J., & Klenk, D. C. (1985) Measurement of protein using bicinchoninic acid. *Analyt. Biochem.* 150, 76–85.
- Şimşekli, Y. (1994). *The fungus exist in the air outside the houses in the different regions of Bursa*. (Master's thesis, Uludağ University).
- Tao, A., & Raz, E. (Eds.). (2015). *Allergy bioinformatics* (8nd ed., pp.1-14). Springer.
- Walker, J. M. (2002). *The Protein Protocols Handbook* (pp. 11-14). Second edition. Humana Press.
- Wójcicka, A. (2014). Changes in pigment content of triticales genotypes infested with grain aphid Sitobion avenae (Fabricius)(Homoptera: Aphididae). *Acta Biologica Cracoviensia. Series Botanica*, 56(1).

Author Information

Mustafa KOROGLU

Gaziantep University
Osmangazi, Üniversite Blv., 27310 Şehitkamil/Gaziantep,
Turkey
Contact e-mail: mustafakoroglu.k@gmail.com

İbrahim Halil KILIC

Gaziantep University
Osmangazi, Üniversite Blv., 27310 Şehitkamil/Gaziantep,
Turkey

Işık Didem KARAGOZ

Gaziantep University
Osmangazi, Üniversite Blv., 27310 Şehitkamil/Gaziantep,
Turkey

Mehmet OZASLAN

Gaziantep University
Osmangazi, Üniversite Blv., 27310 Şehitkamil/Gaziantep,
Turkey

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