The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2021

Volume 12, Pages 119-122

ICRETS 2021: International Conference on Research in Engineering, Technology and Science

Extraction and Purification of the Potential Allergen Proteins from Aspergillus Fumigatus

Cemil Akin UNAL
Gaziantep Universty

Ibrahim Halil KILIC
Gaziantep Universty

Bekir CAKMAK
Gaziantep Universty

Abstract: Allergic diseases are an important public health problem today and are increasing rapidly around the world. According to the research conducted by the World Allergy Organization (WAO), %22 of the world population is struggling with allergic diseases. In our country, one out of every five citizens complains of at least one form of allergy, and it is the third most common chronic disease in children. Some of the allergic diseases develop suddenly and are life threatening. The diagnosis and treatment of allergic diseases is one of the most expensive diseases in our country and in the world. It is triggered by the production of IgE(Immunoglobulin E) in the body against antigens in individuals sensitive to allergen substances. Allergens that play a role in the development of allergic diseases are protein molecules that stimulate IgE synthesis. Pollen, fungus and house dust mites are the most common allergens due to their widespread presence in nature. The presence of fungal spores in the atmosphere for a long time with the effect of air flow increases the possibility of people coming into contact with these allergens. It is very important for health to determine which factors individuals have allergies to. Among the current diagnostic methods; respiratory function tests, skin tests with allergens (prick tests, interdermal tests, patch tests), serum total IgE determination and procovation tests. All of the existing tests used in the identification of allergic diseases in our country are of foreign origin and are imported. One of the fungi that cause allergies is Aspergillus fumigatus from the genus Aspergillus. In our study, we aimed to purify Aspergillus fumigatus allergen proteins and produce allergens that can be used in allergy diagnosis methods. For this purpose, protein isolation and purification of Aspergillus fumigatus, which was cultured and multiplied in SDA (Sabouraud Dextrose Agar) medium in the laboratory, was performed with 2 different methods. The protein amount of the product obtained was determined by the BCA(Bicinchoninic Acid) method. In our studies, it was determined that the protein content of the extract prepared with chloroform/methanol solution was higher than the protein amount of the extract prepared with ethanol solution.

Keywords: Allergy, Aspergillus fumigatus, Allergen protein, Fungal allergy, BCA (Bicinchoninic Acid)

Introduction

Allergic diseases are a very important health problem that affects many people from all age groups, significantly affecting the quality of life of patients and their families, and the number of patients increases over time. With the acceleration of industrialization, there has been a parallel increase in atmospheric pollution, which has led to an increase in allergic diseases around the world. Allergy can be transmitted genetically as well as can occur

with environmental factors. These environmental factors can be biological or chemical. Among the most important biological factors causing allergies are mites, pollen and fungal spores (ASAN 2002).

The habitats of fungi have very large areas. They can be found in the atmosphere, soil, aquatic ecosystems, dead plants and animals, although they are usually seen in damp places such as kitchens and bathrooms and in corners that cannot be ventilated, in foods such as vegetables, fruits and bread. Fungal spores carried by air in the atmosphere can cause very serious diseases when inhaled by humans and animals. Fungi that cause the most allergic diseases are Aspergillus, Alternaria, Penicillium and Cladiosporium genera (CORSİCO 1998).

Fungi take part in the ecosystem as saprophytes, but they can also survive as parasites on some living things. There are spores in the atmosphere, which are the reproductive structures of many fungi. Fungi love moist environments and the number of spores in the atmosphere increases when it rains. These fungal spores cause allergic reactions in humans and animals (DURUGBO 2013).

Allergen substances are antigens that form their specific IgE (Immunoglobulin E) antibody and bind to this antibody. Allergic Bronchopulmonary Aspergillosis (ABPA) is an important lung disease that affects more than 4 million people worldwide, caused by the allergens of Aspergillus fumigatus. An increase in Aspergillus-specific serum IgE (Immunoglobulin E) is observed in the serum of patients with ABPA (KURUP 2000). The average size of Aspergillus fumigatus spores can be 2.0 μm to 3.0 μm. Due to such a small size, the spores can easily become airborne. In addition, as Aspergillus fumigatus has the capacity to reproduce very easily and quickly, the human respiratory tract is always at risk of contracting ABPA infection (LATGE 2001).

It is very important for health to determine which factor individuals are allergic to. Among the available diagnostic methods; respiratory function tests, skin tests with allergens (Prick tests, interdermal tests, patch tests), serum total IgE determination and procovation tests are included. All of the current tests used in the identification of allergic diseases in our country are of foreign origin and are imported. In our study, we aimed to purify Aspergillus fumigatus allergen proteins and produce allergens that can be used in allergy diagnosis methods. For this purpose, the protein amount of Aspergillus fumigatus cultured and grown in SDA (Sabouraud Dextrose Agar) medium in the laboratory was determined by BCA (Bikinchoninic Acid) method.

Method

Preparation of *A. Fumigatus* Extracts

Aspergillus fumigatus strain 9197 was purchased commercially and propagated in our laboratory. Aspergillus fumigatus spores inoculated on SDA medium were incubated for 15 days at 25°C. After morphological examination, samples were collected with the help of sterile wooden cotton swab. The collected mycelium was transferred into chloroform/methanol (2:1) solution and mixed in a falcon tube at 4°C for 24 hours on a magnetic stirrer. The degreased mycelium was left to dry with blotting paper at room temperature (25°C) for 24 hours. The powdered materials were mixed in the prepared phosphate buffered saline (PBS) (1:50 g/ml) at 4°C for 72 hours on a magnetic stirrer. The solution was centrifuged at 5000 rpm for 30 min at 4°C. The supernatant was taken and centrifuged again at 5000 rpm for 30 min at 4°C. The supernatant was dialyzed in 25% PBS at 4°C for 6 hours and then dialyzed again in distilled water at 4°C for 6 hours. The solution was frozen at -20°C for 72 hours and then lyophilized. Protein extraction of dialysis and lyophyse samples was performed according to the extraction method of Ziwei Li et al. (2018). Protein concentrations were determined by extracting the obtained extracts into SDS buffer solution.

Determination of Total Protein Concentration

The total protein concentration of the mushroom extracts was determined using the bicinchoninic acid (BCA) method proposed by Smith et al. A commercially purchased BCA Macro Assay was used to determine protein concentration. BCA analysis was performed following the protocol recommended by the manufacturer (Walker, 2002). The protein amounts of the mushroom extracts prepared in the study were determined with the help of the standard graph drawn with bovine serum albumin (BSA) standards.

Results and Discussion

In our study, Aspergillus fumigatus proteins, one of the allergenic fungi, were extracted with 2 different extraction protocols. Extracted protein amounts were measured by the BCA method. The protein amounts of the mushroom extracts prepared in the study were determined with the help of a standard graphic drawn with bovine serum albumin (BSA) standards.

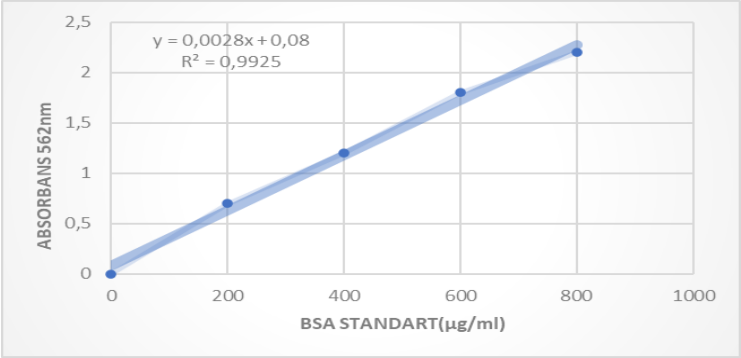

Figure 1. Standard graph used to determine protein concentration

Table 1. Total protein concentrations of Aspergillus fumigatus extracts measured by BCA method

Alergen name	Absorbance Measurements	Absorbance Average	Protein concentration (µg/mL)
Aspergillus fumigatus (Chloroform/Methanol)	0,802/0,791/0,801	0,798	228
Aspergillus fumigatus (Ethanol)	0,774/0,767/0,775	0,772	218

In our study, Aspergillus fumigatus proteins, one of the allergenic fungi, were extracted. Extracted protein amounts were measured by the BCA method. As a result of the data obtained, the total protein amount of Aspergillus fumigatus was 228 µg/mL according to the Chloroform/Methanol protocol and 218 µg/mL to the ethanol protocol. The amount of protein obtained from the Chloroform/Methanol protocol was higher than the amount of protein obtained from the ethanol protocol. The protein amounts obtained in previous studies of Aspergillus fumigatus (Kahlert 1996) and the protein amounts obtained in our study support our study.

Conclusion

In recent years, cases of allergies caused by fungi have been increasing. For this reason, studies on the determination of allergen proteins of fungi, which are common in nature, have gained importance. In our study, Aspergillus fumigatus extracts, which is one of the allergenic fungi and used in allergen kits, were prepared and their protein concentrations were determined. The data obtained from this study form the basis for the production of alternative domestic kits to the imported kits used in the diagnosis and treatment of allergy patients with subsequent studies.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

References

Asan, A., Sen, B., & Sarica, S. (2002). Airborne fungi in urban air of Edirne city (Turkey). *Section Botany*, *57*(1), 59-68.

Klironomos, J. N., Rillig, M. C., Allen, M. F., Zak, D. R., Pregitzer, K. S., & Kubiske, M. E. (1997). Increased levels of airborne fungal spores in response to Populus tremuloides grown under elevated atmospheric CO2. *Canadian Journal of Botany*, *75*(10), 1670-1673.

Corsico, R., Cinti, B., Feliziani, V., Gallesio, M. T., Liccardi, G., Loreti, A., ... & Zerboni, R. (1998). Prevalence of sensitization to Alternaria in allergic patients in Italy. *Annals of Allergy, Asthma & Immunology*, *80*(1), 71-76.

Guneser S, Atici A, Koksal F, & Yaman A. (1994). Mold allergy in Adana, Turkey. *Allergol Immunopathol 22*(2), 52-4.

Yurdakulol, E., & Cansaran, D. (2004). Tohumsuz bitkiler I laboratuvar kılavuzu. 74, Basılmamış.

Durugbo, E. U., Kajero, A. O., Omoregie, E. I., & Oyejide, N. E. (2013). A survey of outdoor and indoor airborne fungal spora in the Redemption City, Ogun State, south-western Nigeria. *Aerobiologia*, *29*(2), 201-216.

Kurup, V. P., Banerjee, B., Hemmann, S., Greenberger, P. A., Blaser, K., & Crameri, R. (2000). Selected recombinant Aspergillus fumigatus allergens bind specifically to IgE in ABPA. *Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology*, *30*(7), 988-993.

Latgé, J. P. (2001). The pathobiology of Aspergillus fumigatus. *Trends in microbiology*, *9*(8), 382-389.

Walker, J. M. (2002). SDS polyacrylamide gel electrophoresis of proteins. In *The protein protocols handbook* (pp. 61-67). Humana Press.

Smith, P. E., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M., ... & Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Analytical biochemistry*, *150*(1), 76-85.

Kahlert, S. (1996). *Identification and characterisation of a specific extracellular component of Aspergillus fumigatus.* [Doctoral dissertation], University of Surrey.

Author Information

Cemil Akın UNAL
Gaziantep Universty
Osmangazi, Üniversite Blv., 27310
Şehitkamil/Gaziantep, Turkey
Contact e-mail: *cmlaknunl@gmail.com*

Ibrahim Halil KILIC
Gaziantep University
Osmangazi, Üniversite Blv., 27310
Şehitkamil/Gaziantep, Turkey

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

To cite this article:

Unal, C.A., Kilic, I.H., & Cakmak, B. (2021). Extraction and purification of the potential allergen proteins from Aspergillus Fumigatus. *The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 12*, 119-122.