Session 1: Molecular Aerobiology

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Microbial fragments as air contaminants – release mechanisms and biological activity

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Traditional aerobiological methods usually focus on quantitative and qualitative evaluation of microbial vegetative cells, spores or conidia. Such approach neglects both the immunological reactivity of small fragments of their structural elements and the emission strength of microbial colonies. Hence, the proper exposure assessment should contain not only comprehensive identification of microbial contaminants but, if possible, credible measure of the emission strength of their source as well. In this presentation, fungal and actinomycetal propagule (fragments and spores) aerosolization process from microbiologically contaminated surfaces will be described. The problem description will include the following components: testing the release of fungal and bacterial propagules from different types of surface materials, evaluation of the effects of air velocity and vibration of the surface on the propagule release process, and quantitative assessment of fragment and spore release as a function of time, as these propagules are exposed to air currents and vibration. Application of high performance analytical instruments and techniques (such as optical and ultrafine particle counters, scanning electron microscope, monoclonal antibodies, and cell culture) to quantitative evaluation and qualitative characterization of the emission of submicrometric propagules will also be discussed. A newly designed and constructed aerosolization chamber showing its ability to measure "the microbial source strength" using two (perpendicular and swirling) aerosolization methods will be presented as well. This new instrument is believed to be a useful tool to assess the maximal microbial contamination level in indoor environment and could help to overcome the limitations of traditional bioaerosol sampling methods.

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Alt a 1 – a unique β-barrel protein

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Human kind has been troubled by allergies since the beginning of written history. Allergic reactions to certain substances were known even in ancient Egypt or Greece. Asthma, rhinitis, rash, digestive problems are the main ailments related to allergies. Asthma is one of the most common and one of the most serious diseases of the

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airways caused by inhaled allergens. The course of asthma may be acute or chronic, and asthma attacks may be caused by exposure to pollen, mold, animal saliva, animal dander, feces of the house dust mites, cockroach particles, as well as certain non-allergenic substances. The elucidation of the Alt a 1 allergen structure and an attempt to understand its molecular structure may contribute to the development of proper immunotherapy and, thus, reduce asthma symptoms in people affected by the allergy to Alt a 1. The Alt a 1 allergen comes from black mold (*Alternaria alternata*), which is common in outdoor environment in mild climate zones and is a major health hazard for humans. X-ray crystallography was used to determine the structure of Alt a 1 by using a custom-designed set of crystallization conditions. An initial Alt a 1 model was determined by the application of a $Ta_6Br_{12}^{2+}$ cluster and single-wavelength anomalous diffraction. Bioinformatical analyses were used to compare the Alt a 1 sequence and structure with that of other proteins. They revealed the structure of Alt a 1 as a unique dimeric β-barrel protein comprising 11 β-strands and forming a „butterfly-like" dimer linked by a single disulfide bond with a large (1345 Å^2) dimer interface. Intramolecular disulfide bonds are conserved among Alt a 1 homologs. Solving it defined a new protein family with unknown function found exclusively in fungi. It is the first step for further research and identification of structure-function relationship, which can lead to better understanding of its allergenic properties and the development of the new forms of immunotherapy for Alt a 1 sensitive patients.

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Detection of the main allergen on *Alternaria alternata* (Alt a 1) in different air fractions

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Alternaria sp. is one of the most important allergenic fungi. Fungal allergens are produced by both spores and hyphae. The airborne hyphal fragments, much smaller than fungal spores, may reach lower respiratory tract. However, fungal allergy forecasts are solely based on information related to fungal spore concentrations in the air. The main aim of this study was to determine concentrations of the main allergen of *Alternaria alternata* (Alt a 1) in three air fractions, i.e. PM>10μm, 2.5>PM>10μm, and 0.12>PM>0.25μm.

Alternaria spores and Alt a 1 were collected in 2016 by volumetric spore trap of Hirst design and by three-stage high volume Chemvol impactor (stage I PM>10μm, stage II 2.5>PM>10μm, and III 0.12>PM>2.5μm) located in Poznań (Western Poland). Concentrations of Alt a 1 were determined by ELISA. Alt a 1 levels were compared with fungal spores data collected by both samplers. Correlations between spores and Alt a 1 in three air fractions were determined by Spearman rank correlation test.

Daily concentrations of spores collected by Hirst-type spore trap and Chemvol impactor were statistically significant ($r^2>0.9$; $p<0.05$). The highest number of spores, similarly as Alt a 1 was observed in 2.5-10 air fraction. In III stage, the amounts of Alt a 1 and spores were extremly low. The correlation between daily

levels of *Alternaria* spores and Alt a 1 was statistically significant.

The vegetative hyphal fragments might be a source of allergens; however, the significant correlation between fungal spores and Alt a 1 found in three stages of Chemvol impactor suggests that fungal spores play the main role in fungal allergy. Nevertheless, the quantitative analysis of fragmented mycelium would be a desirable practice in routine aerobiological monitoring.

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DNA-based detection and back-trajectory modelling allow tracking fungal pathogens of the genus *Leptosphaeria* transported in air

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Molecular detection of living organisms may serve as a very useful tool in aerobiological studies, making it possible to add important data on fungal and plant species and even their genetic variants transported by air, as spores and pollen grains. DNA-based monitoring of fungal species in the air, supplemented with the back-trajectory modelling allows migration studies of airborne fungi, and it is the only solution when microscope-based spore identification is impossible, due to high similarities of fungal spores of different taxons.

The study was done in Szczecin in 2006-2015, with the use of Hist-type spore trap. We investigated two specific events relating to long distance transport of fungal spores, identified as *Leptosphaeria* sp. The fungi were identified using dual-labelled fluorescent probes that were targeted to a *β*-tubulin gene fragment of either *Leptosphaeria maculans* or *L. biglobosa*; these two fungi are serious pathogens of oilseed rape (*Brassica napus*). Spore identification by Real-Time PCR techniques capable of detecting minute amounts of DNA of selected fungal species was combined with back-trajectory analysis, allowing the tracking of past movements of air masses using the HYSPLIT model.

DNA-based monitoring with the use of species-specific probes allowed us to track the aerial movements of two important fungal pathogens of oilseed rape, which have identical spore shape and size. We found that the spores detected in two studied events both belonged to *L. biglobosa*. We also demonstrated that, on the studied occasions, the spores originated from the Jutland Peninsula.

This is the first successful attempt to combine analysis of back-trajectories of air masses with DNA-based identification of economically important pathogens of oilseed rape in Europe. The timing of *L. biglobosa* ascospore dispersal in the air detected in our studies was unlikely to result in the infection of winter oilseed rape grown as a crop plant. However, the fungus could infect other cruciferous crops and weeds. It is a direct proof that pathogens of cultivated and wild plants can be transported to new areas, even from sources located far away.

Artemisia pollen allergens expression profile

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Artemisia species are wind-pollinated perennial weeds, which belong to the family Asteraceae. Their pollen is one of the most important aeroallergens in Central Europe. In Poland, *Artemisia* is considered the third most important source (after birch and grasses) of allergenic pollen. The prevalence of pollinosis to *Artemisia* allergens is approximately 10-15%. In Wielkopolska, three species of *Artemisia* are commonly found: *A. vulgaris*, *A. absinthium* and *A. campestris*. In the pollen of *A. vulgaris*, six allergenic proteins that are responsible for the most allergy symptoms in Poland in July and August were identified. They have been named sequentially: Art v 1, Art v 2, Art v 3, Art v 4, Art v 5 and Art v 6. *Artemisia* allergens belong to different protein families and play distinct biological roles. Some of them are released in stress conditions, others are involved in signaling pathways or act as transfer molecules.

We found homologues of allergens from *A. vulgaris* in pollen of *A. absinthium* and *A. campestris*. The main aim of our study was to determine and compare the expression profiles of genes encoding allergenic proteins in inflorescences containing pollen in different stages of its development and in mature pollen of three *Artemisia* species. To show the time of production and the levels of mRNAs encoding of these proteins during pollen development, we used precise and sensitive real-time PCR method.

We determined the genes expressed on the highest level on subsequential stages of pollen development. We also observed that the most immunogenic allergens are expressed in anther cells which suggests that they are transported to pollen as mature proteins. On the basis of the obtained expression profiles, we could classify three genes as "late" pollen genes. This means that they are transcribed only in mature pollen; there is no noticeable expression of them in the earlier stages of pollen grain development. Among three examined species, the highest normalized expression of allergens we recorded in *A. vulgaris* pollen.

The performed experiments shed new light on the studies of *Artemisia* allergens. Additionally, we proved that not only *A. vulgaris* pose a threat to allergy risk, and this is important to take other *Artemisia* species into account in research related to diagnosis and therapy.

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Identification of pollen grains by spectroscopic methods

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