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-omics technologies bridging food and environmental sciences

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Book of Abstracts

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Session 1: Omics and the environment

Invited lecture

Highly improved method for in-depth post-translational modification profiling: example of Timothy grass (Phleum pratense) pollen proteomes from polluted and preserved environments

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Field-realistic exposure studies provide the most relevant assessment of the effects of the intensity and diversity of urban and industrial contamination on pollen structure and allergenicity. The significance of in-depth post-translational modification (PTM) studies of pollen proteomes, when compared with studies on other aspects of pollution and altered pollen allergenicity, has not yet been determined; hence, little progress has been made within this field.

Therefore, we created a comprehensive approach for the comparison of pollen from polluted and environmentally preserved areas. To examine the effects of long-term, in vivo pollen exposure to multiple source pollutants, *Phleum pratense* (Timothy grass) pollen samples were collected along a regional road in Kruševac, central Serbia. This road experienced moderate traffic and was located near a chemical plant that produces fertilizers. Pollen samples from this location were compared with pollen samples collected from a rural, environmentally preserved area over two consecutive pollination seasons. We combined the quantitative comparison of proteome expression profiles from in-solution and 2D gels with unrestrictive in-depth quantitative PTM profiling using high resolution tandem mass spectrometry and the PEAKS 8.5 Suite platform. This was followed by

quantitative IgE enzyme-linked immunosorbent assays (ELISA) and one dimensional (1D) IgE immunoblots that were probed with the sera of grass pollen allergic patients and healthy control subjects from Serbia. In addition, elemental compositional analyses of Timothy grass pollen samples from both locations, and the surface grain structure and SPP releasing potential (including total protein and phenolic content), were assessed.

An increased phenolic content and release of sub-pollen particles was found in pollen samples from the polluted area, including a significantly higher content of mercury, cadmium, and manganese. Antioxidative defense-related enzymes were significantly upregulated and seven oxidative PTMs were significantly increased (methionine, histidine, lysine, and proline oxidation; tyrosine glycosylation, lysine 4-hydroxy-2- nonenal adduct, and lysine carbamylation) in pollen exposed to the chemical plant and road traffic pollution sources. Oxidative modifications affected several Timothy pollen allergens; Phl p 6, in particular, exhibited several different oxidative modifications. The expression of Phl p 6, 12, and 13 allergens were downregulated in polluted pollen, and IgE binding to pollen extract was substantially lower in the 18 patients studied, as measured by quantitative ELISA.

Quantitative, unrestricted, and detailed PTM searches using an enrichment-free approach was used for the first time to map extensive modifications in the pollen allergome, which was shown to reflect the increased environmental oxidative stress, primarily caused by increased content of heavy metals in pollen. With some modifications, this PTM profiling approach is suitable for exploring the oxidative stress effects in any proteomic source in a quantitative in-depth manner, thus enabling further data-driven research.

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