P015 Searching for phosphoproteins that are crucial for tobacco pollen activation *in vitro*

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Tobacco mature pollen has an extremely tough cell wall and a desiccated cytoplasm. It rehydrates *in vivo* on a stigma tissue, and develops into the rapidly-growing pollen tube. During this rehydration process, the stored mRNA transcripts have been de-repressed, and protein synthesis has been started. Furthermore, such metabolic switch is also likely to be regulated by post-translational modifications of the present proteins, namely via phosphorylation since it was shown to be the most dynamic post-translational regulation that is likely to play a significant regulatory role in many cellular processes. The application of various enrichment techniques is usually of key importance in order to increase the number of identified phosphoproteins because of the fact that only a minor part of proteins is phosphorylated at a time in a cell and they can coexist there with their native forms. This enrichment can be performed either at the level of intact phosphoproteins or at the level of peptides produced by a protein fragmentation.
In our studies, metal oxide/hydroxide affinity chromatography (MOAC) with aluminium hydroxide matrix was applied in order to enrich phosphoproteins from mature pollen and *in vitro* 30-min activated pollen crude protein extracts. The enriched fraction was separated by both 2D-GE and gel-free approaches with subsequent mass spectrometric analyses. To broaden the number of phosphorylation sites identified in the 139 phosphoprotein candidates, titanium dioxide phosphopeptide enrichment from trypsinized mature pollen crude extract was performed in parallel. In total, 58 phosphorylation sites present in the above phosphoprotein candidates were assigned.