PHOSPHOPROTEIN ENRICHMENT FROM SOLUBLE AND MICROSOMAL FRACTIONS OF GRAPEVINE (VITIS VINIFERA CV. GAMAY) CELL CULTURE USING METAL OXIDE AFFINITY CHROMATOGRAPHY (MOAC)

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Phosphorylation is one of the most prominent post-translational protein modifications in living cells and its investigation is of key interest in the field of proteomics. However, the frequently low stoichiometry of phosphorylation makes phosphoproteins harder to detect and identify. To overcome this problem, pre-fractionation methods of total cellular proteins are highly desirable.

In this study, we have performed a phosphoenrichment at protein level by metal oxide affinity chromatography using $Al(OH)_3$ as a metal binding matrix for phosphate group capture [1]. Here, a strategy based on phosphoprotein enrichment by MOAC followed by a separation using a gel-based approach and phosphoprotein detection by specific fluorescence staining is shown. Phosphoprotein enrichment and subsequent protein separation using a gel-based approach (1D SDS-PAGE/2-DE) avoids the problem of complex protein mixtures analysed by gel-free technologies. Positive-stained proteins were identified by LC-MS/MS and phosphorylation sites were analysed using the automatic detection of neutral loss scan for H_3PO_4 [2].

The strategy performed in this study includes a pre-fractionation of total protein extract previous of phosphoprotein enrichment to analysed separately soluble and microsomal proteins fraction, thus maximizing phosphoproteome coverage. A novel phosphoprotein enrichment using MOAC as for soluble protein fraction [1] have been successfully achieved for microsomal fraction.

[1] Wolschin F, Weckwerth W. Plant Methods. 2005 Nov 1;1(1):9.
[2] Wolschin F, Wienkoop S, Weckwerth W. Proteomics. 2005 Nov;5(17):4389-97.

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