

Quantitative Proteomic Analysis of Anti-cancerous Scopularide production by *Scopulariopsis brevicaulis* LF580

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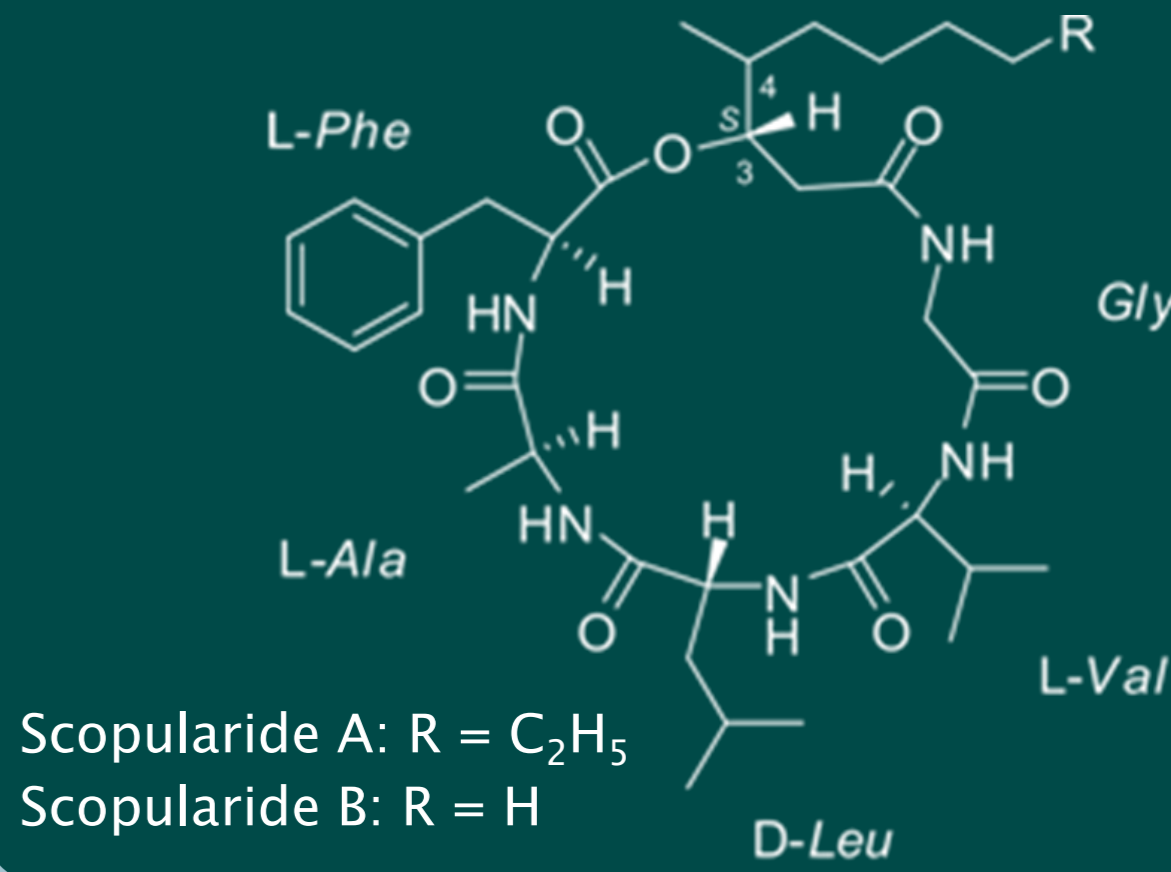
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Introduction

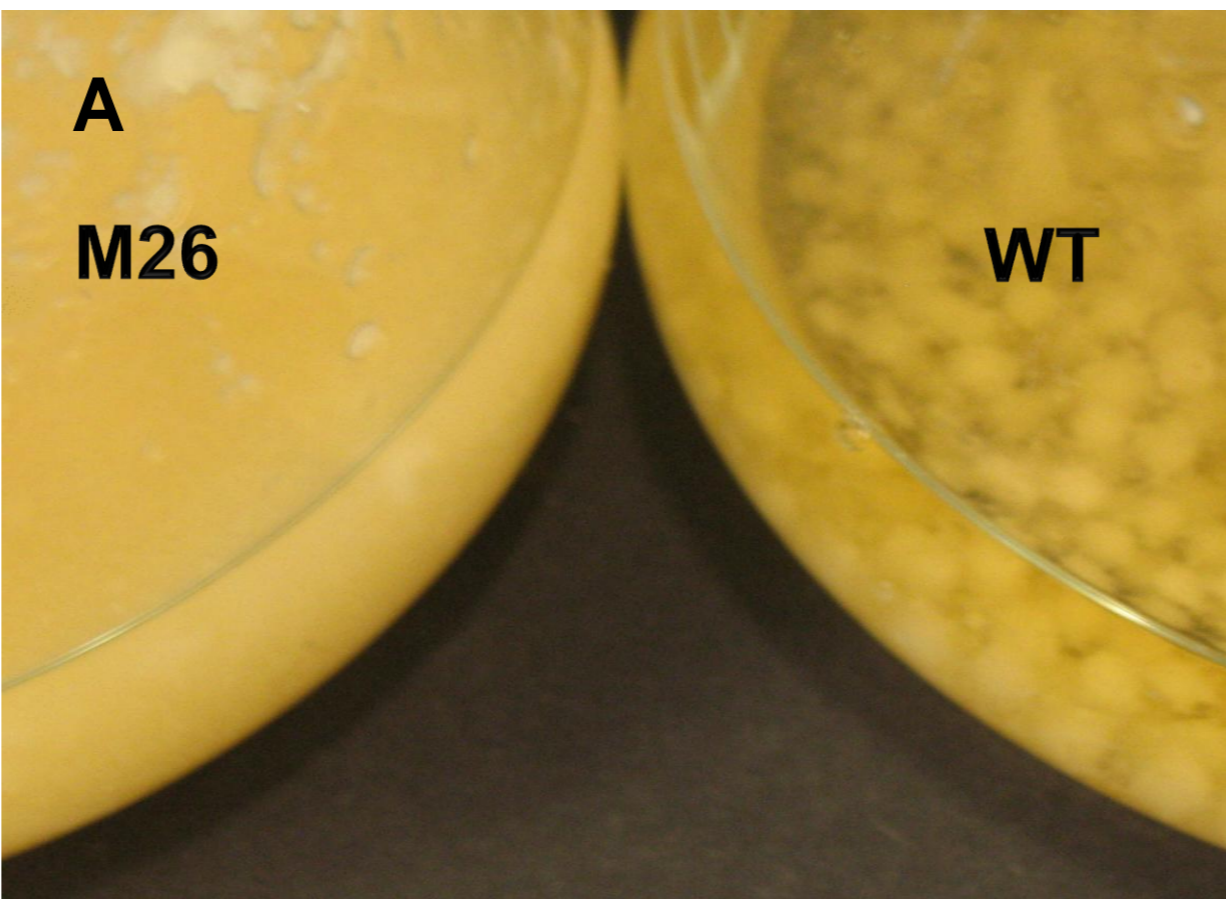
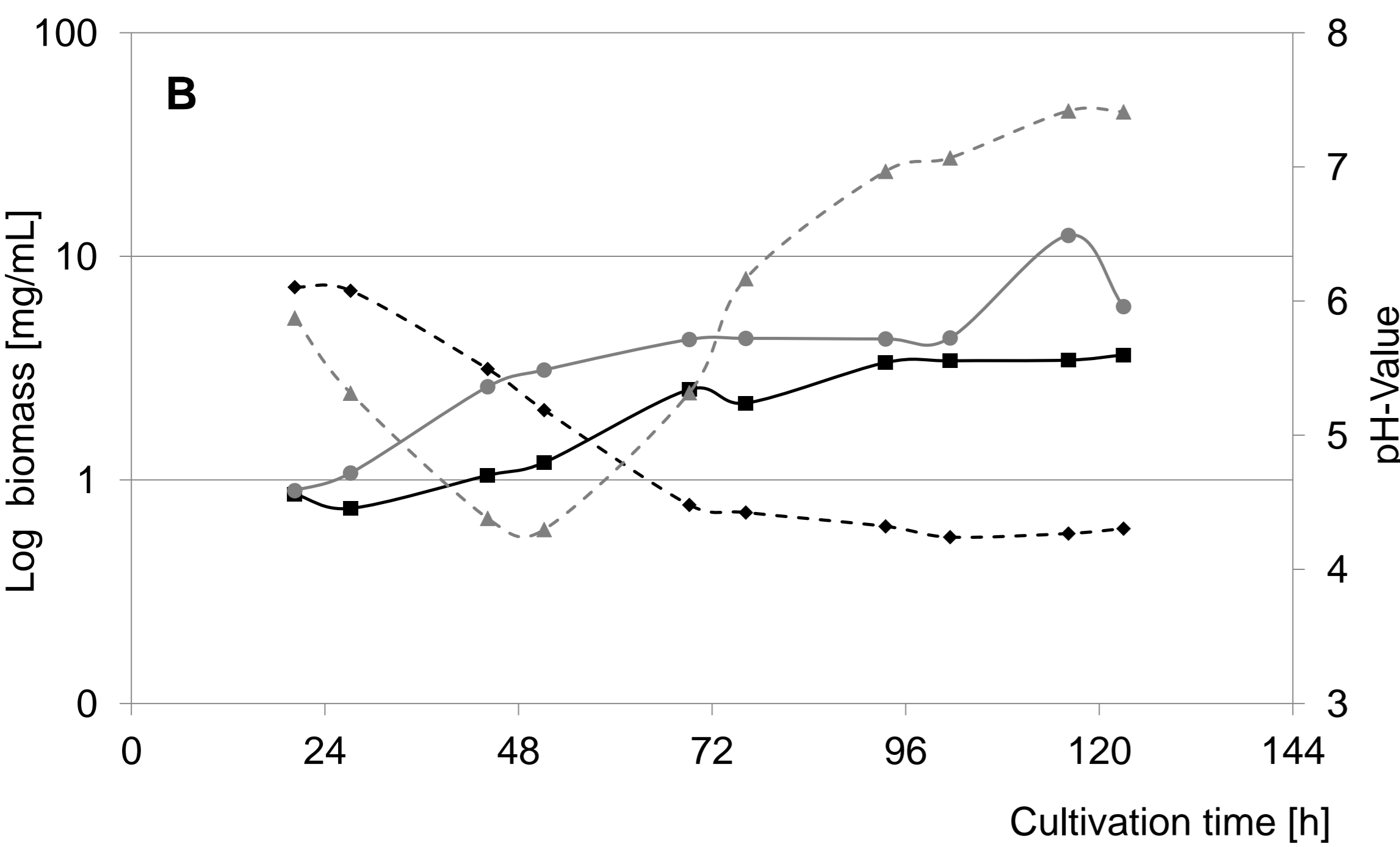
Within the EU-project MARINE FUNGI (EU FP7, 265926), the marine fungus *Scopulariopsis brevicaulis* LF580, isolated from the marine sponge *Tethya aurantium*, was selected for genome analysis as well as for several optimization process including random UV mutagenesis, with respect to the production of the bioactive cyclodepsipeptides. In order to gain a deeper understanding of the hitherto uncharacterized molecular mechanisms underlying the production of scopularide A and B by *Scopulariopsis brevicaulis* LF580, we applied quantitative proteomics to compare the proteomes extracted from the wild type strain and the mutant strain M26. Using iTRAQ-based quantitative proteomic approach will provide novel biological information useful for the targeted optimization of scopularide production by *Scopulariopsis brevicaulis* LF580.

Scopularide A/B [1]:



Cultivation-based characterization of mutant strain LF580-M26

The UV-mutant strain M26 of *Scopulariopsis brevicaulis* LF580 exhibited a modified morphological growth (A) in liquid medium, as well as a faster biomass production resulting in a higher yield of scopularide A and B (B).

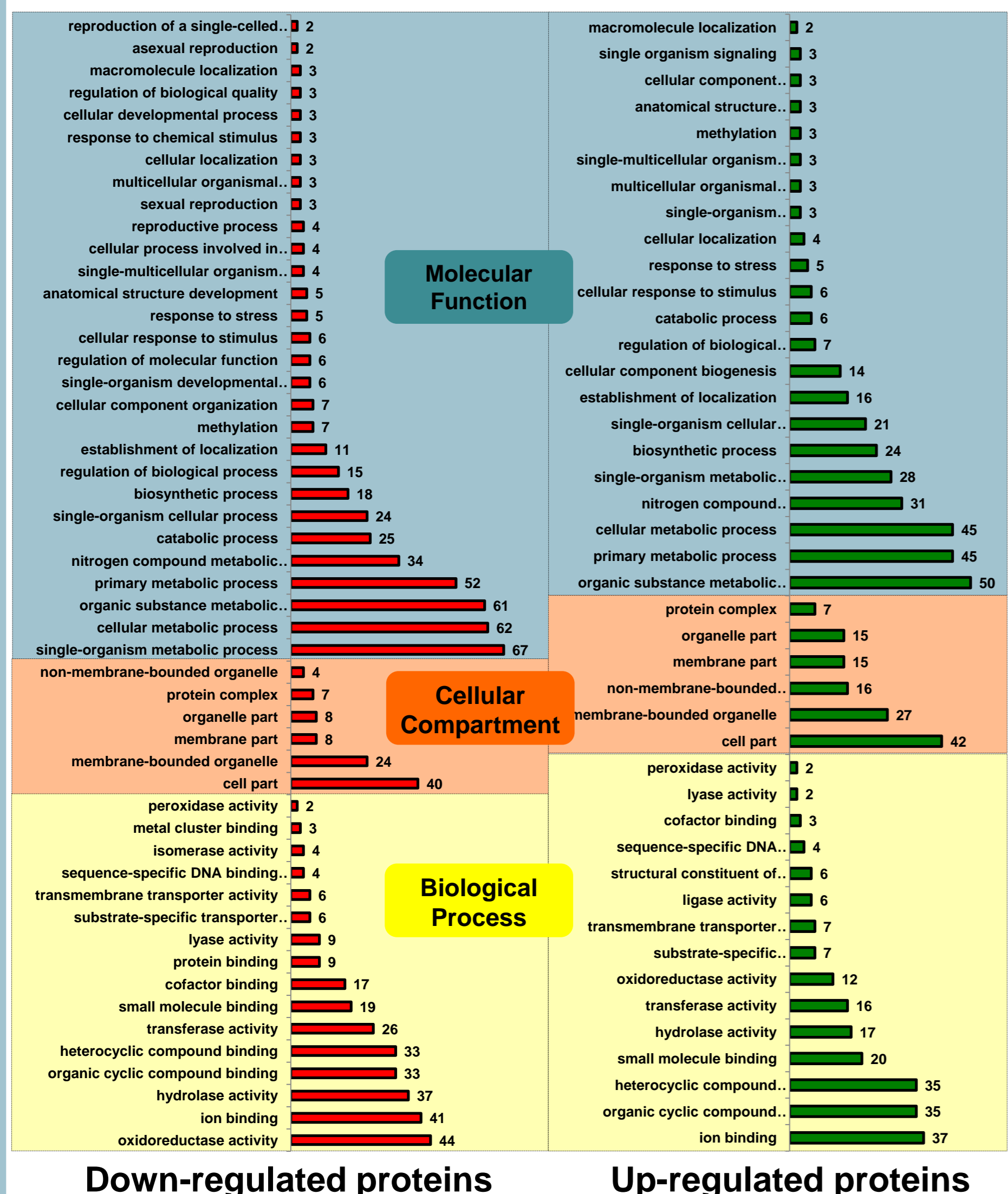


A: Pellet formation and shape of WT and M26: Cultivation in 300 mL Erlenmeyer flasks containing 100 mL WSP30-medium
B: Time-series of WT and M26: carried out in 300 mL Erlenmeyer flasks containing 100 mL WSP30-medium: Determination of biomass of WT (—■—) and M26 (—●—), as well as pH of WT (---◆---) and M26 (---▲---) in triplicates.

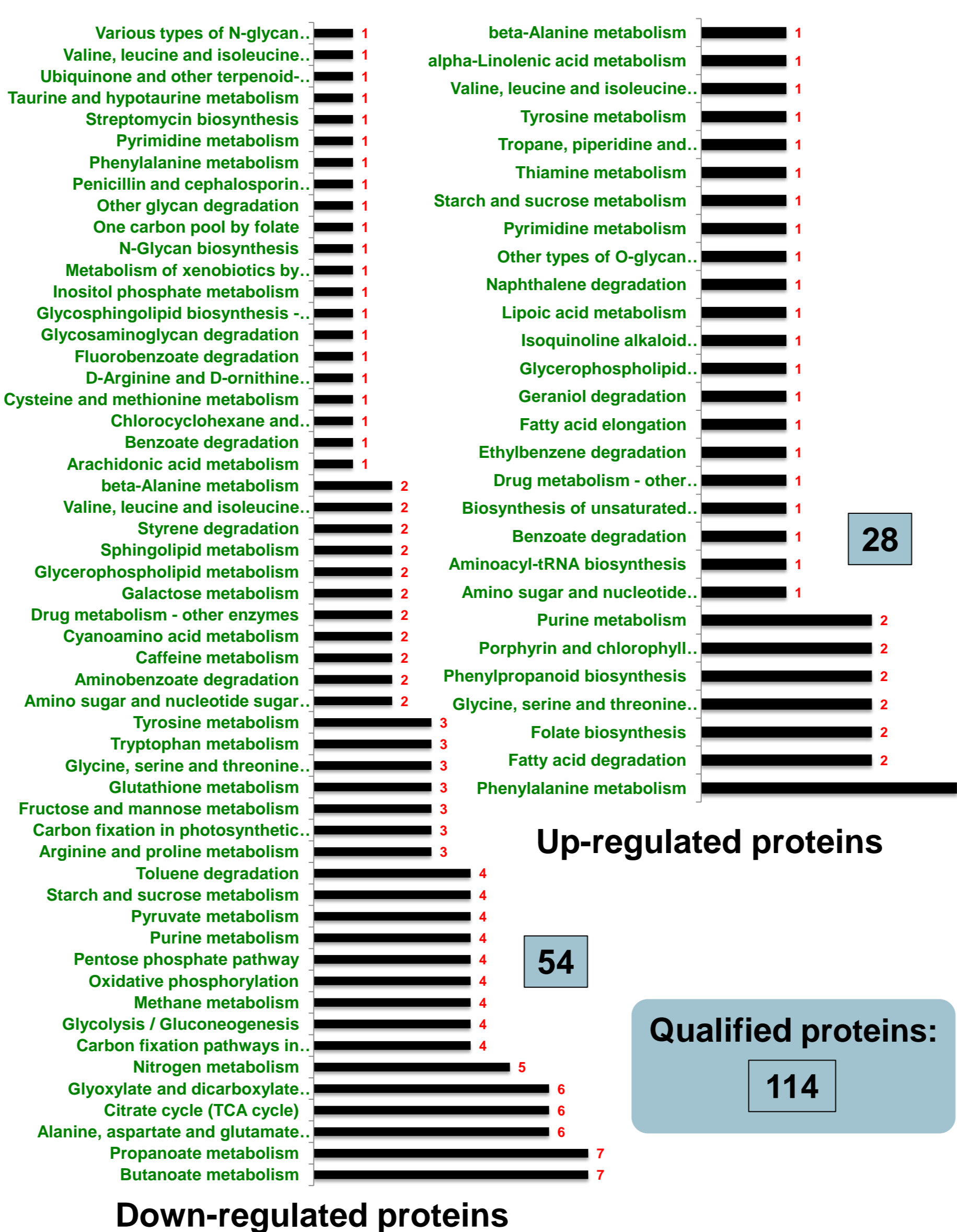
Gene ontology and KEGG pathway analyses

Summary of gene ontology (GO) terms in three categories (I) and of KEGG pathway analyses (II) for down- and up-regulated proteins, based on iTRAQ labelling. Annotational analyses were performed using BLAST2GO tool.

I: Gene ontology (GO) terms of three categories



II: KEGG pathways analyses



Cultivation in triplicates of WT and M26

Two methods of cell disruption: liquid nitrogen and beads

Protein extraction

iTRAQ labelling

HILIC separation

nano-LC MS/MS analysis [2]

Data analysis: MASCOT-SEQUEST / annotated genome of LF580

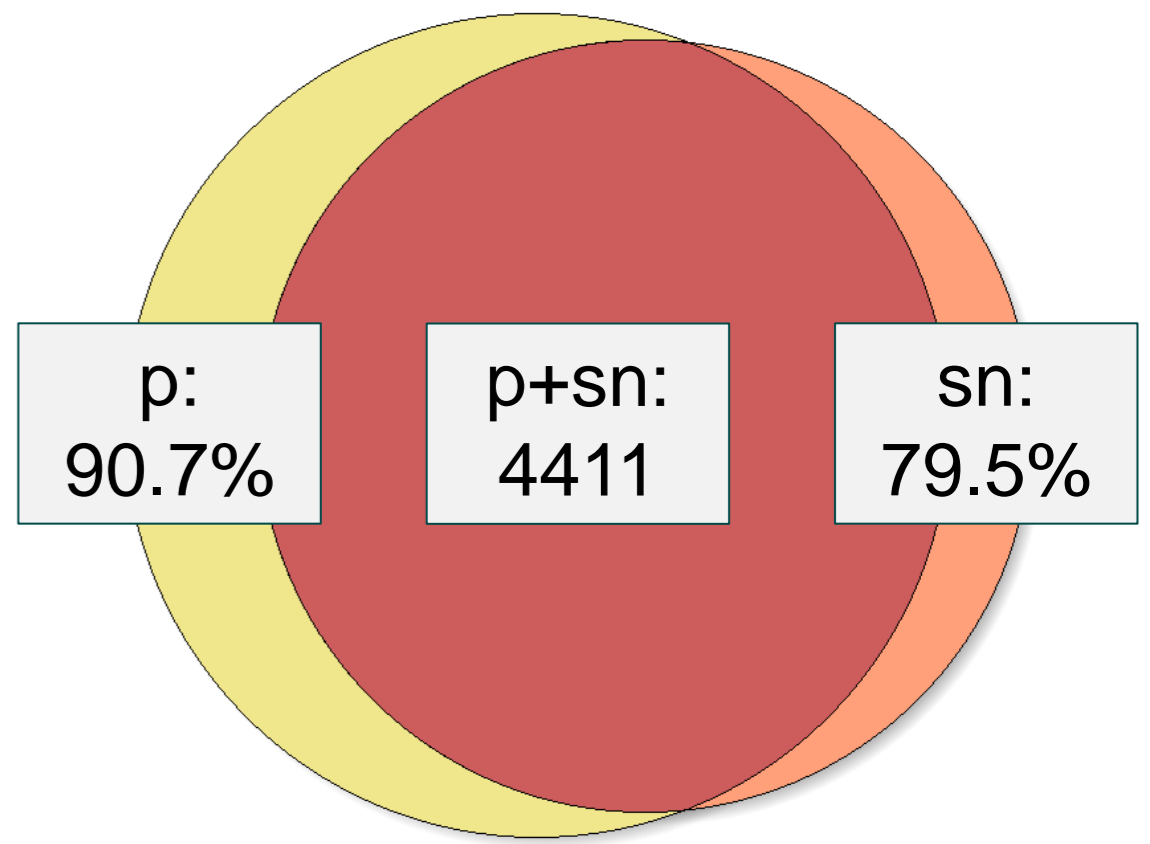
Identification of significant regulated proteins by statistical analysis

Gene ontology and KEGG pathway analyses of regulated proteins

Protein extraction

Two cell disruption methods were compared, based on grinding in liquid nitrogen and disruption using beads. Adapted protein extraction method using several washing steps, as well as the use of 8 M urea, lead to an increase of the number of identified unique proteins (4759).

Extracting grade of the liquid nitrogen (N) based extraction method:



Integration of further solubilisation steps combined with a predigest into the extraction protocol lead to an increase in coverage. Hence the analyses comprised both, the easy to solve proteins (sn) as well as the precipitating proteins (p).

Conclusion

The development of an efficient protein extraction method enabled us to extract increased and comparable amounts of proteins from the *Scopulariopsis brevicaulis* LF580 strain and its UV mutant strain (*Scopulariopsis brevicaulis* LF580-M26).

Gene ontology analysis of the differentially regulated proteins in the mutant strain as compared to the wild type strain demonstrated an enrichment of proteins and pathways involved in growth and metabolism.

KEGG pathway analysis demonstrated that the down-regulated proteins were associated with enzymes of the TCA cycle, butanoate and propanoate metabolism, and the metabolism of the TCA-derived amino acids glutamate and aspartate, whereas the up-regulated proteins were associated with the biosynthesis of the oxaloacetate-derived amino acids, serine, glycine, and threonine, fatty acid degradation, and phenylalanine metabolism.

iTRAQ labelling: 3526 unique proteins were identified using iTRAQ labelling combined with nano-LC-MSMS analysis. 318 (9%) proteins showed a regulation, based on the normalization of the ratio against the median peptide ratio. 5.3% were down-regulated, 3.7% represent an up-regulation.

References

[1] Yu, Z; Lang, G; Kajahn, I; Schmaljohann, R; Imhoff, JF *J. Nat. Prod.* 2008, 71, 1052–1054
[2] Beck HC; Petersen J; Felthaus O; Schmalz G; Morscheck C *Neurochem Res* 2011, 36: 2002-2007