



Integration and regulation of nicotine metabolism in *Paenarthrobacter nicotinovorans* as revealed by proteomic data

Clarkson UNIVERSITY
defy convention

Biochemistry and Proteomics Group
I Love Proteins!

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Overview

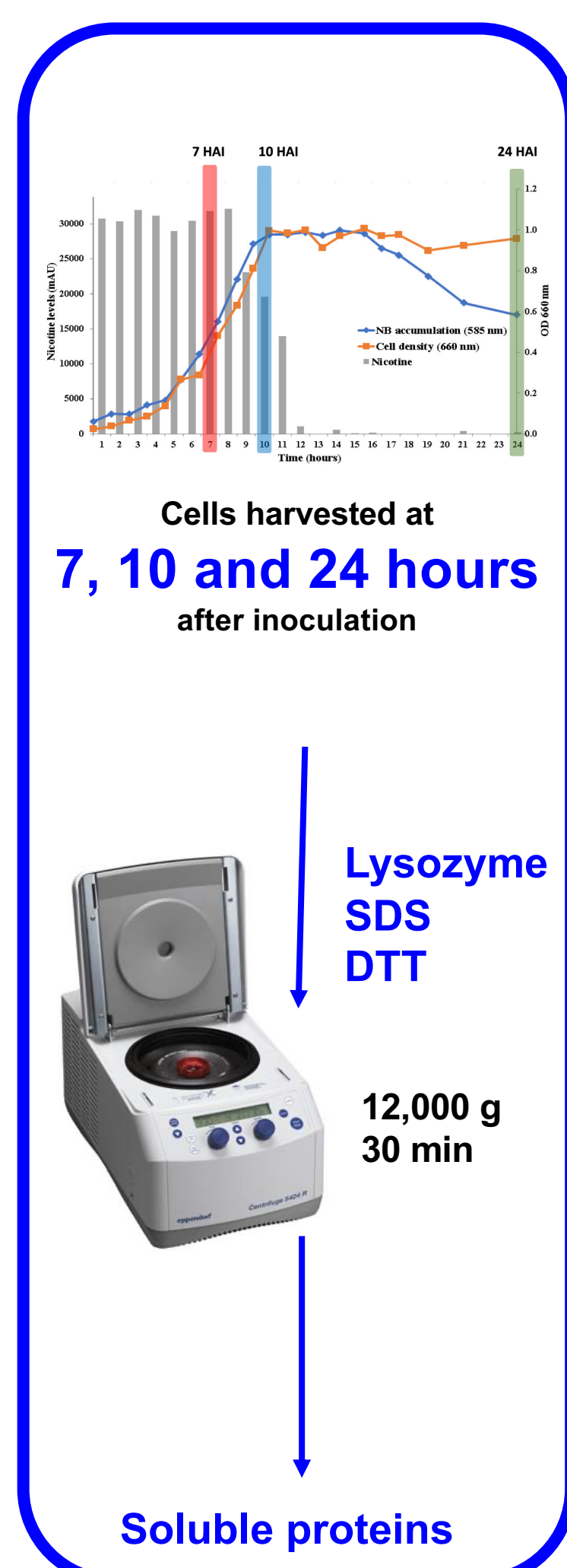
- 915 proteins grouped in 528 non-redundant protein clusters with an FDR of 0.3% were identified using a gel based nanoLC-MS/MS approach;
- proteomics data available PRIDE with the dataset identifier PXD012577.

Introduction

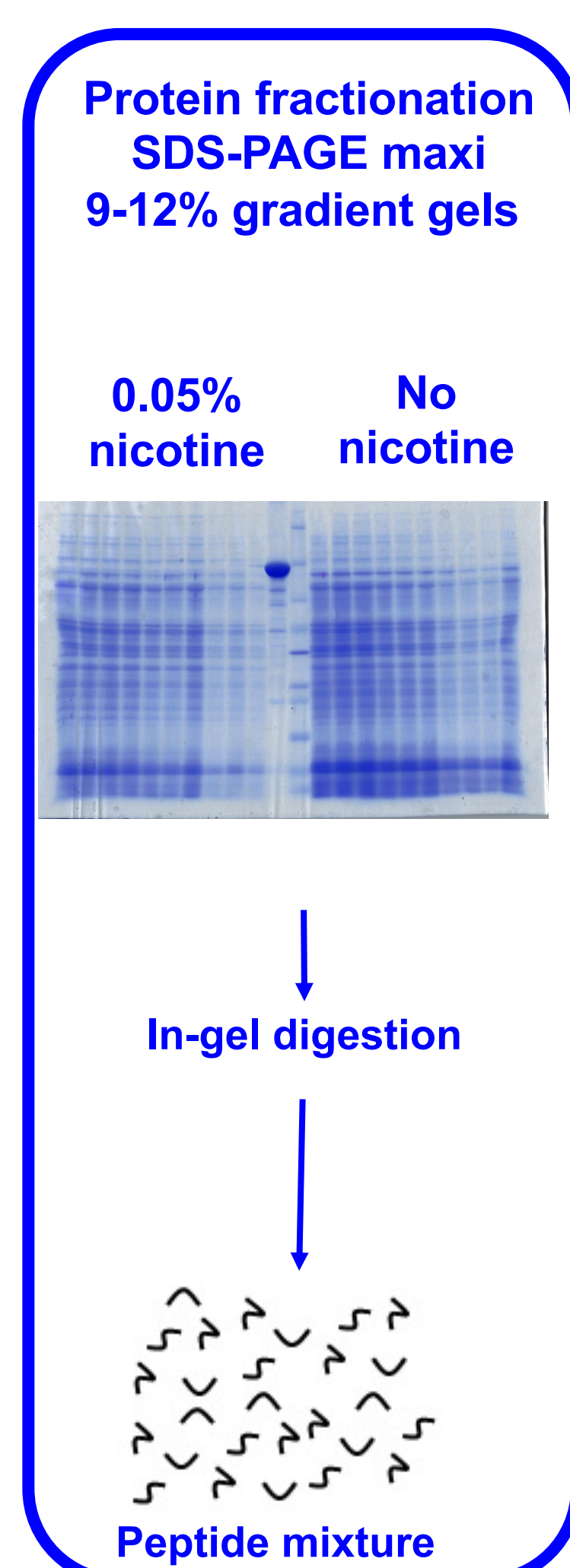
Paenarthrobacter nicotinovorans is a soil Gram-positive nicotine-degrading microorganism (NDM) that harbors a 165 kb pAO1 catabolic megaplasmid. The nicotine catabolic genes on pAO1 have been sequenced, but not all the details on the regulation and interplay of this pathway with the general metabolism of the cell are available. Also, little is known on how the cells cope with the accumulation and toxicity of the resulting nicotine metabolic by-products. Here, we used nanoLC-MS/MS and performed a time-based proteomic study of *P. nicotinovorans* grown in the presence or absence of nicotine.

Methods

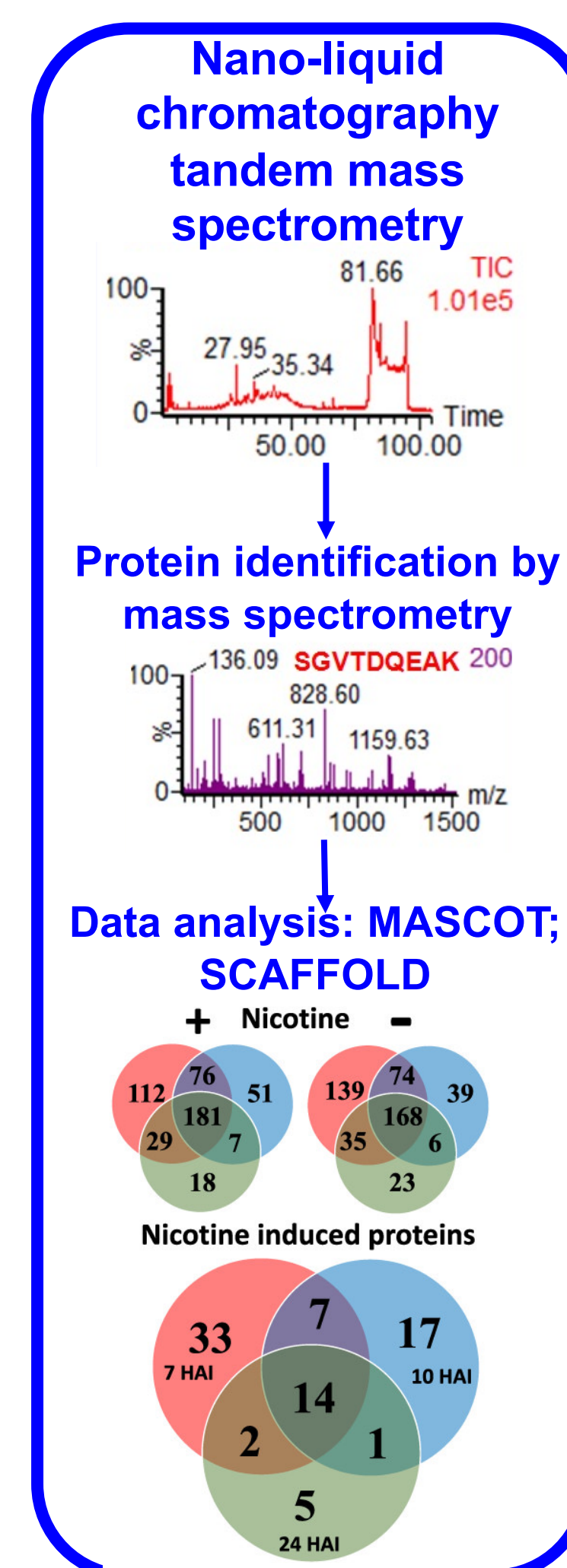
Growth, harvest and cell lysis



Sample preparation

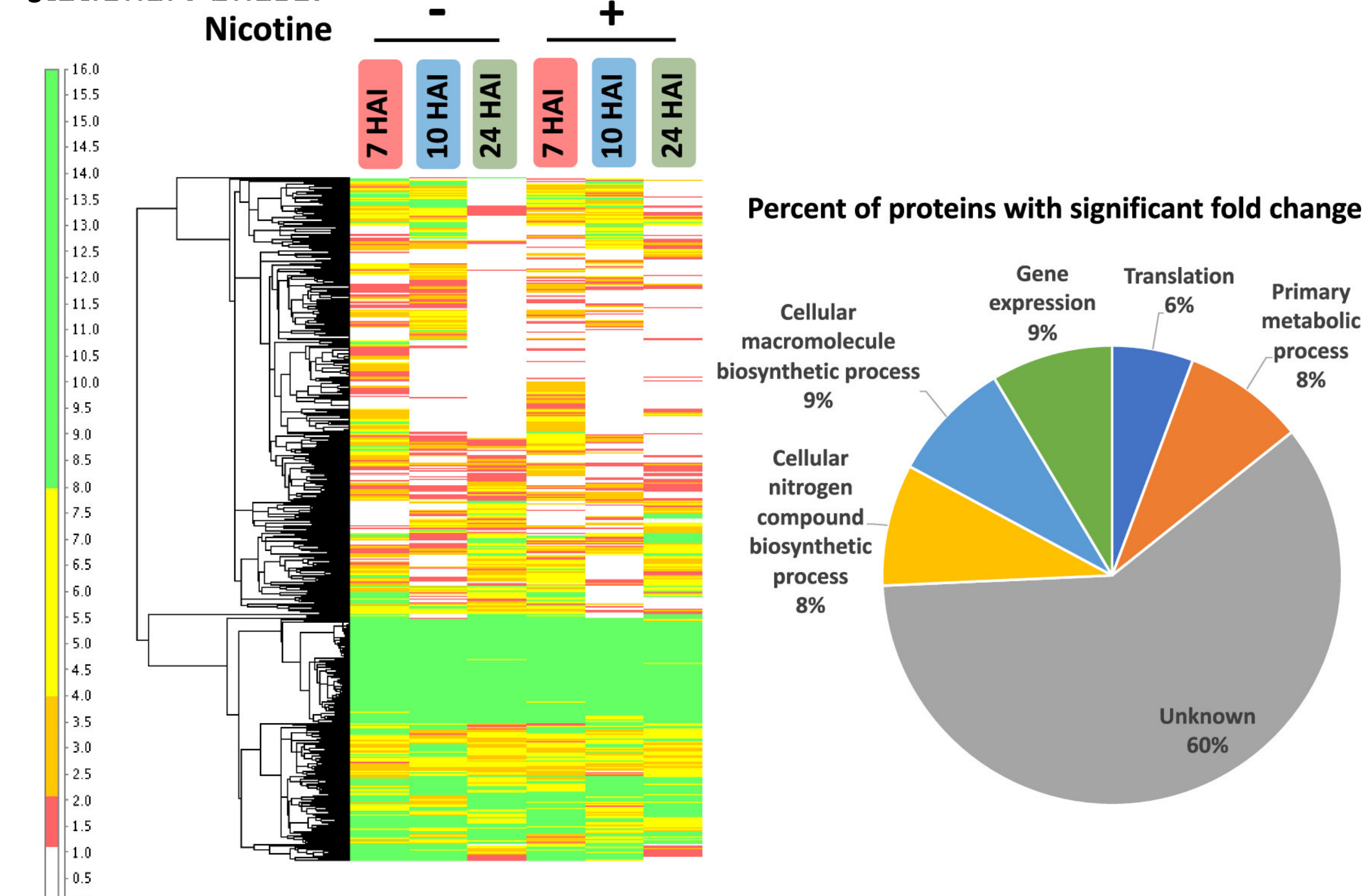


Identification and data analysis

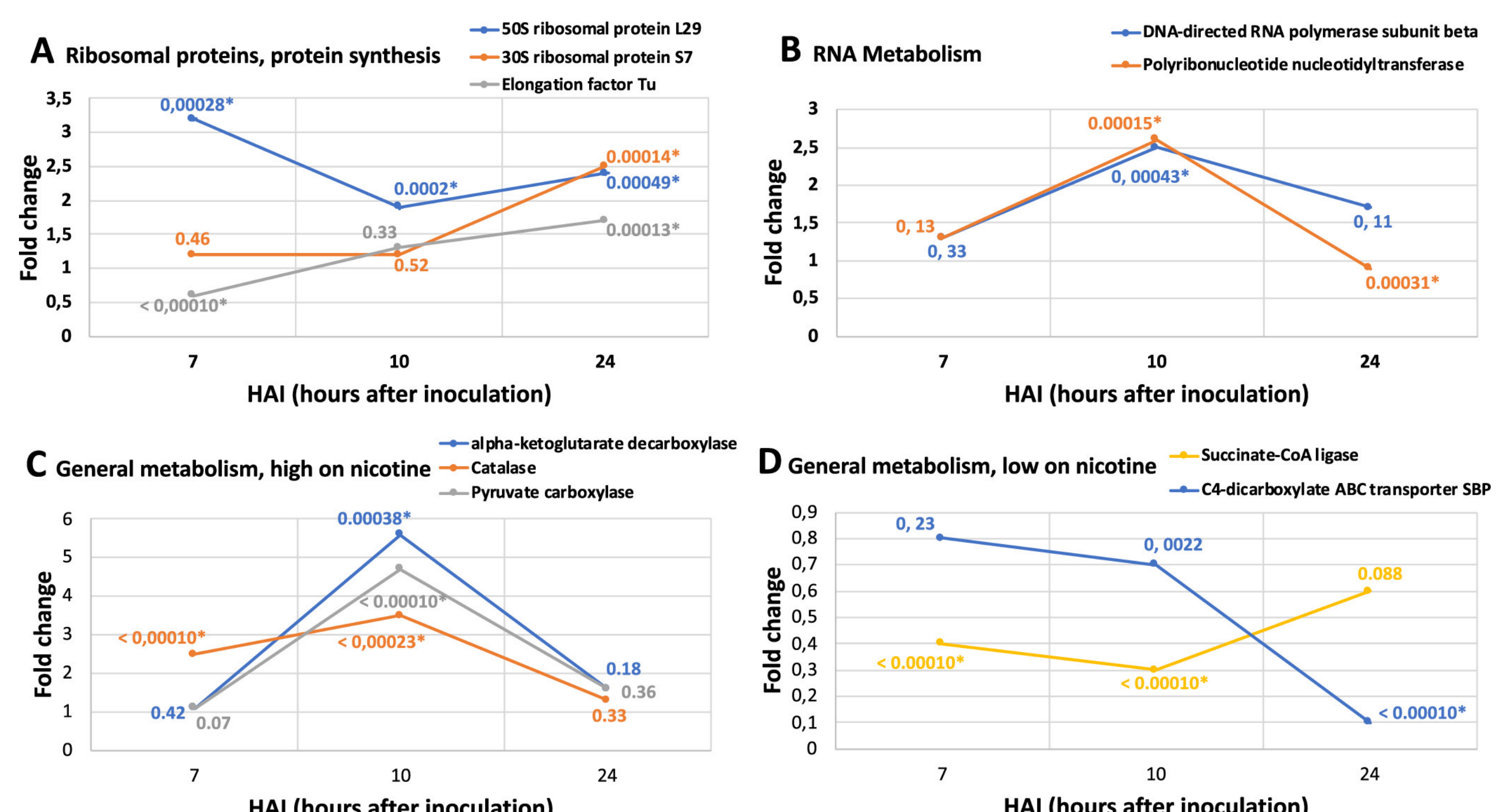


Results

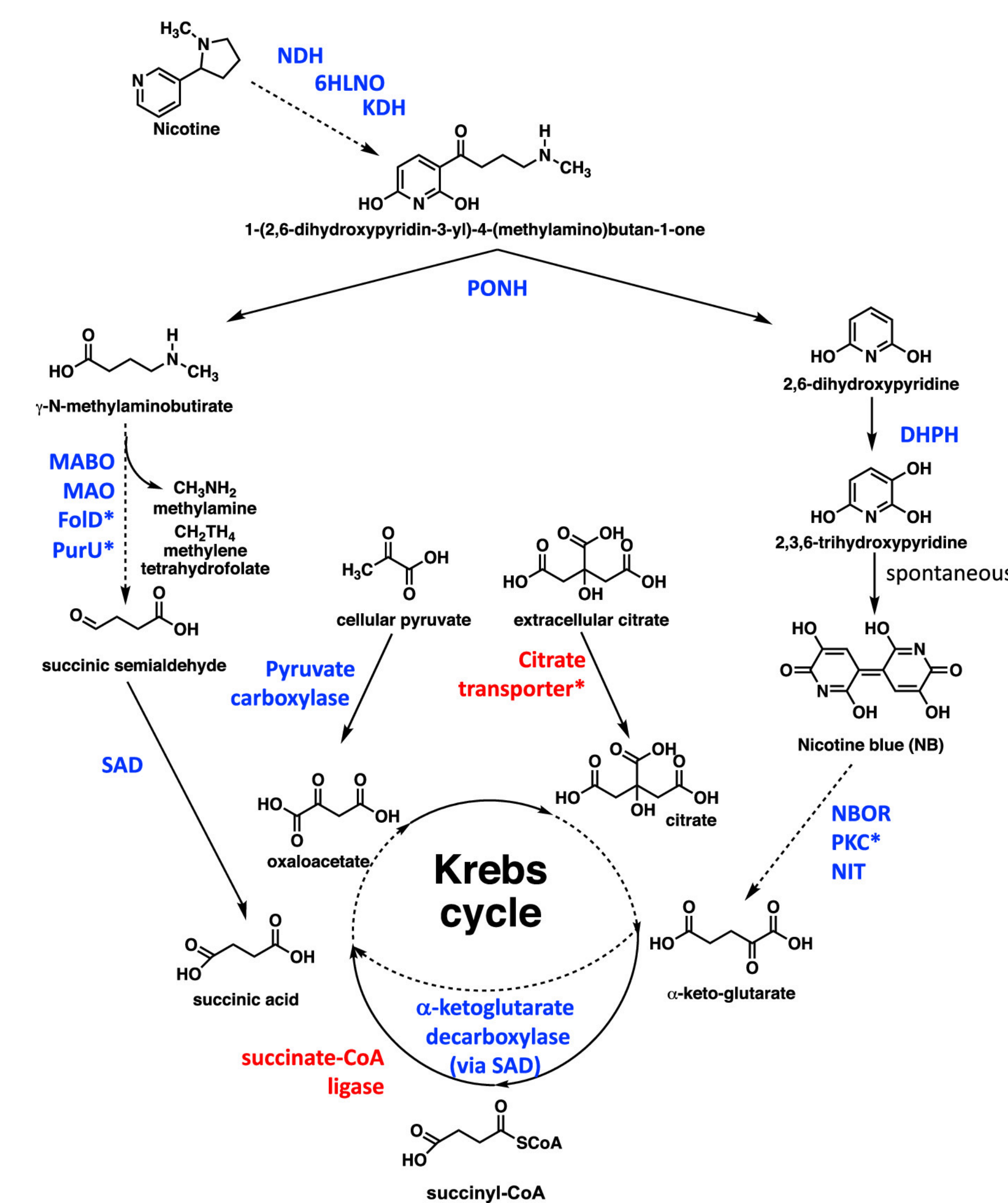
A bottom-up approach based on the MASCOT MS/MS database search algorithm was used to identify a total of 915 proteins grouped in 528 non-redundant protein clusters with an FDR of 0.3%. Our time-course proteomics experiments included analysis of key points in nicotine metabolism: 7 h after inoculation (HAI) which corresponds to the middle log phase; 10 HAI corresponds to early early stationary phase and 24 HAI corresponding to late stationary phase.



Several new chromosome encoded enzymes related to the general metabolism of the cell have been found to be regulated by nicotine and provide an anaplerotic pathway that links the Krebs cycle to the nicotine catabolism in this bacterium.



Our time-course proteomics experiments also allow us to determine the when the Krebs cycle is active and when the nicotine pathway becomes active and when the two of them work together for an efficient energetic metabolism via expression of various proteins through chromosomal-plasmidial gene regulation



Conclusions

- Five new chromosome-encoded enzymes related to the general metabolism of the cell reported here indicate that this is done through anaplerotic pathways, allowing us to understand the bacterial management of energy using the Krebs cycle, nicotine pathway, or both.;
- These experiments can also lead to a better understanding of the pAO1-encoded catabolic pathway of *P. nicotinovorans* and the energy supply-based regulated expression of the plasmidial and chromosomal genes.

References

Mihasan & Brandsch (2016) Microb. Res. DOI:10.1016/j.micres.2016.05.008
 Mihasan et. Al. (2018) Sci.Rep.doi DOI: 10.1038/s41598-018-34687-y
 Aslebagh et. Al. (2016) Electrophoresis . DOI: 10.1002/elips.201600134
 Brandsch R. (2006) FEBS J. DOI: 10.1111/j.1742-4658.2006.05173.x
 Elpiniki et. al. (2016) J Proteomics DOI: 10.1016/j.jprot.2014.08.018

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