Effect of nutrient stress on protein stability in the bioplastic producer Cupriavidus necator – monitoring conformation at proteome level using thermal proteomics

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Cells can respond to environmental insult through coordinated expression changes (mRNA, protein) or via structural modifications to individual proteins that confer functional change (e.g. conformation, allostery, molecular interactions). The former can be assessed using approaches like transcriptomics and expression proteomics, while until recently the latter have been difficult to study en masse. Thermal Proteome Profiling (TPP) assays the thermostability of proteins on a proteome-wide level by measuring the progressive loss to aggregation of proteins subjected to a temperature gradient. The melting behaviour of each protein can be compared under different conditions, permitting the identification of conformation response to the condition. Furthermore, studies have demonstrated that proteins participating in shared pathways and protein complexes can show correlated melting pattern changes. TPP is therefore a promising approach to understanding the global response to environmental changes that may be independent of, or supplementary to, gene or protein expression programmes. Here, we apply the TPP method to a nutrient stress insult in the bioplastic (PHA, poly-hydroxyalkanoate) producing chemolithoautotroph *Cupriavidus necator*. *C. necator* is of intense interest in sustainability studies since it can consume carbon dioxide to produce biofuel. Over 90% of the dry cell weight of *C. necator* can comprise of PHA granules. *C. necator* cells, grown in complete media and in media with reduced nitrogen, were harvested. TPP analysis was implemented using tandem mass tag (TMT) labelling to encode the soluble proteome surviving each of 10 temperature steps (30°C to 100°C). Trypsin-digested samples were then analysed by Orbitrap mass spectrometry and melting curves calculated for >1000 proteins. Proteins showing evidence of conformation change in response to low nitrogen included enzymes involved nitrogen metabolism, as well as central metabolism pathways like the TCA cycle.