Modulation of glycolysis and homolactic fermentation of *Streptococcus thermophilus* energetically discharged cells: dissecting the role of transcription and post-translational modifications

Zanchetta Ylenia, Stefania Arioli, Diego Mora

Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, via Celoria 2, via Mangiagalli 25, 20133, Milan, Italy

S. thermophilus metabolizes lactose via homolactic fermentation. Lactose enters the cell through LacS, is hydrolyzed by β-galactosidase into glucose and galactose, the galactose is exported, and the glucose is converted to lactic acid via glycolysis. To shed light on the regulatory network of glycolysis and homolactic fermentation, cells harvested in the exponential growth phase were divided into aliquots and subjected or not to an ATP and glucose depletion treatment, in presence and absence of rifamycin and chloramphenicol to block transcription and translation. The resulting ATP or glucose depleted biomasses (EdCs) were subjected to comparative metatranscriptomics and metaproteomics analysis, and quantification of (p)ppGpp. The EdCs phenotype showed the lowest levels of (p)ppGpp and an up-regulation of the S10 ribosomal protein gene cluster and purine biosynthesis genes, consistent with the regulatory scenario headed by the stringent response. The metabolic behaviour of those biomasses was then evaluated by activating them with lactose, lactose and urea, in presence or in absence of glycolytic inhibitors. EdCs activated with lactose behaved as biomasses treated with glycolytic inhibitors, displaying no production of L-lactic acid, while exhibiting a higher lactose capacity compared to their control. Data analysis revealed transcriptional regulation of genes and post-translational modification of proteins involved in glycolysis correlated with the yield of glycolysis and lactose intake capacity. Proteomic analysis showed ATP and glucose depletion significantly alters protein lactylation profiles, indicating that addition and removal of lactyl groups on lysine residues are regulated at post-translational level by acetyltransferases and deacetylases.