Proteome analysis of the purine stimulon from Lactococcus lactis - DTU Orbit (10/11/2019)

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A comparative expression proteome analysis was carried out by analyzing differential expression patterns of pulse-labelled proteins on two-dimensional gels under standard conditions and during purine nucleotide starvation, followed by mass spectrometric identification of regulated proteins. Based upon the expression patterns, three stimulons could be identified in Lactococcus lactis subsp. cremoris. The Psu proteins (purine starvation up-regulated) had increased synthesis during purine depletion in a purine auxotroph. Among these proteins were enzymes of the purine biosynthesis pathways (PurE, PurS, PurM, PurL), and enzymes involved in the generation of C1 units (GlyA, Fhs). C1 units are primarily required for purine biosynthesis. Upon analysis of the nucleotide sequence preceding the structural genes for these proteins in the L. lactis IL1403 genome sequence showed that all contained PurBox-Pribnov box structures resembling the PurR activated promoters for the purDEK and purCSQLF operons. Most, and possibly all members of the Psu stimulon are thus members of the PurR regulon. Five Psu proteins could not be identified. The second stimulon, the Psd stimulon (purine starvation decreased), whose members are down-regulated during purine depletion, contained proteins related to protein synthesis (PpsB, EF-TS, trigger factor), or to GTPases (FtsZ, EF-TS); or are involved in energy metabolism (GapB, CcpA). No common regulatory elements could be found for members of this stimulon. Two Psd proteins escaped identification. The last, Dcu (decoynine up-regulated), stimulon contained proteins whose synthesis escaped the severe general depression during inhibition of the GMP synthetase by decoynine. This regulon was comprised of mostly glycolytic enzymes (fructose bisphosphate aldolase, enolase, pyruvate kinase) and translation elongation factors (GTPases: EF-TU, EF-G). Two Dcu proteins could not be identified. Out of 28 proteins subjected to mass spectrometry, 19 could be readily identified despite the fact that only the genome sequence of a strain of L. lactis subsp. lactis was available. The two subspecies share about 85% sequence identity, comparable to the genetic distance between Escherichia coli and Salmonella typhimurium. A success rate of 68% indicates that it may be feasible to perform proteomics based upon genomic sequences of relatives outside the genus.

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