# Omics-Driven Analysis of *Clostridium ljundhalii* Disentangles the Complexity of Energy Conservation in Autotrophic and Heterotrophic Growth Conditions

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**Project goals: We aim to enrich the knowledge base for *C. ljungdahlii* to use it as a model for studying carbon fixation and biofuel production. We use genome-wide translational, transcriptional, transcription start sites, RNA polymerase binding sites, proteomics and other analysis to advance our understanding of energy metabolism and regulatory networks in order to improve the predictability of the ME model. Ultimately, the optimized ME-model will guide future strain design for production of valuable biocommodities and biofuels.**

The availability of an elaborate metabolic model has already placed *C. ljungdahlii* as "the organism of choice" for the study of carbon fixation, acidogenesis and biofuel production. Here we carried out genome-wide ribosome profiling and RNA-seq experiments under two autotrophic (H2:CO2 and CO) and one heterotrophic (fructose) growth conditions to understand the energy requirements for growth. In all conditions studied, the correlation between ribosome profiling and RNA-seq was surprisingly high with Spearman's $\rho$ ranging from 0.86-0.88, suggesting that gene expression is mostly regulated at the transcriptional level.

To study genes that are significantly regulated at the translational level, we filtered outlier genes that had either high or low translational efficiency (TE), such that the Spearman's correlation between ribosome profiling and RNA-seq is increased to 0.9 when they are removed from the total datasets. Among those outliers, we show that the genes encoding the RNF complex have significantly low TE in heterotrophic growth and that they are highly regulated at the translational level. Furthermore, we classify genes into 25 functional categories and based on the growth conditions, we analyzed the differential prioritization of translational and transcriptional resources per metabolic category and the subcategories within. We also report differential distribution of low and high TE genes across conditions with regard to metabolic categories.

Finally, we determined a subset of differentially translated and transcribed regulators that are likely to be involved in the regulatory switch between autotrophic and heterotrophic growth modes. First, we generated a two-way blast map of transcription factors (TFs) from all sequenced acidogenic, solventogenic and pathogenic Clostridia. Then we generated a hierarchical cluster map based on the conservation of each TF. Interestingly, TFs either differentially translated in heterotrophic or autotrophic growth are highly conserved in acetogenes and these are considered to be the growth condition switch regulators. On the other hand, TFs highly conserved across all Clostridia were not differentially expressed in *C. ljungdahlii*, suggesting they are essential for the regulation

of housekeeping genes.