Regulation of acetate metabolism by pH in *Escherichia coli*

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Project Goals: The goal of this project is to determine how protein acetylation affects metabolism in bacteria. Lysine acetylation is a common post- translational modification that eukaryotes, archaea, and bacteria employ to regulate protein activity. Multiple studies have recently shown that lysine acetylation predominantly targets metabolic enzymes – in fact, most metabolic enzymes are subject to lysine acetylation. We hypothesize that bacteria employ lysine acetylation as a global mechanism to regulate metabolism in response to their energy and redox status. Our previous work suggests that lysine acetylation may be an attractive and innovative target for metabolic engineering. We are investigating how lysine acetylation affects bacteria metabolism. The significance of this work is that it will address a fundamental gap in our understanding of bacterial metabolism and identify new approaches for overcoming the problems associated with the production of advanced biofuels.

During growth on excess sugars, the state of growth for most fermentation, *Escherichia coli* will produce acetate due to overflow metabolism. Following consumption of the sugar, *E. coli* will then reassimilate the extracellular acetate. Acetate metabolism is closely connected to protein acetylation, because acetyl phosphate, an intermediate in acetate metabolism, serves as the principal acetyl-group donor in lysine acetylation.

In the project, we investigated extracellular acetate production and reassimilation during growth at neutral and acidic pH's. We found that pH does not affect the production of extracellular acetate during growth on glucose. However, we found that *E. coli* is unable to reassimilate the extracellular acetate during growth under acidic conditions (pH < 7). These results demonstrate that acidic pH's inhibit the ability of *E. coli* to consume – but not produce – extracellular acetate.

To identify the mechanism, we investigated a number of mutants in which the different pathways for acetate metabolism were selectively deleted. These mutants also failed to reassimilate acetate under acidic growth conditions. In addition, we investigated the transcriptional regulation of the acetate metabolic genes and found that their expression was not sensitive to pH. We also investigated the impact of pH on the reversibility of the AckA/Pta pathway using purified enzymes. Much of acetate produced by *E. coli* during growth on glucose is produced via the reversible AckA/Pta pathway. We found lower pH drove acetate in the direction of consumption and inhibited its production in both proteins. This result demonstrates that differences in equilibrium acetate from AckA/Pta cannot explain the pH-dependent reassimilation of acetate. Our current hypothesis is that pH-dependent reassimilation of acetate is due to the repression of the glyoxylate bypass during growth in acidic conditions. We are currently testing this hypothesis.

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