

Increasing Growth Yield and Decreasing Acetylation in *Escherichia coli* by Optimizing the Carbon-to-Magnesium Ratio in Peptide-based Media.

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Project Goals: The goal of this project is to determine how protein acetylation affects metabolism in engineered microorganisms. 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 fuel production in engineered microorganisms. 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.

N^ε-lysine acetylation is a posttranslational modification that occurs within all three domains of life. The acetylation reaction occurs through the donation of an acetyl group from a donor molecule onto a susceptible lysine of a protein or peptide. This modification neutralizes the positive charge of the lysine side chain and increases its size. Acetylation of residues required for catalytic function can render an enzyme inactive. Additionally, neutralization of the positive charge can disrupt salt bridges necessary for protein-protein interactions. In *E. coli*, acetylation is known to be catalyzed by two mechanisms. One, the canonical enzymatic mechanism, utilizes the only known lysine acetyltransferase, YfiQ, to catalyze the donation of the acetyl group of an acCoA molecule onto a lysine. The other and more predominant mechanism employs acetyl phosphate (acP), the intermediate of the acetate fermentation (AckA-Pta) pathway, to donate its acetyl group onto proteins non-enzymatically. Therefore, conditions that promote acetate fermentation invariably lead to protein acetylation.

Previously, our lab and others have found that *E. coli* grown in carbon excess leads to high acetylation levels due to the production of acetate^{1,2}. While studying protein acetylation in buffered tryptone broth supplemented with glucose (TB7/glucose), we observed that *Escherichia coli* did not fully consume glucose prior to stationary phase. However, when we supplemented this medium with magnesium, the glucose was completely consumed during exponential growth with concomitant increases in cell number and biomass but reduced cell size. Similar results were observed with other sugars and other peptide-based media, including lysogeny broth. Buffering was found to be necessary for the full magnesium growth yield increase because without buffering, acidification of the medium due to acetate excretion inhibited growth. Magnesium also limited cell growth for *Vibrio fischeri* and *Bacillus subtilis* in TB7/glucose.

Finally, magnesium supplementation reduced protein acetylation. Based on these results, we conclude that growth in peptide-based media is magnesium limited. We further conclude that magnesium supplementation can be used to tune protein acetylation without genetic manipulation. These results have the potential to reduce potentially deleterious acetylated isoforms of recombinant proteins without negatively affecting cell growth.

References

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2. Kuhn, M.L., et al., Structural, kinetic and proteomic characterization of acetyl phosphate-dependent bacterial protein acetylation. PLoS One, 2014. 9(4): p. e94816.

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