

Impact of the *bolA* Gene on Cellulose Production in the Acetic Acid Bacterium
Komagataeibacter xylinus

Elizabeth Dickey¹, Lindsey Zimmerman¹, Paul Schweiger, PhD¹

¹University of Wisconsin-La Crosse, Department of Microbiology

Cellulose is the most abundant polymer in the world. It is commonly associated with plant cells but is also synthesized by bacteria, such as *Komagataeibacter xylinus*. Bacterial cellulose is especially valued as a wound dressing in burn centers. Unlike plant cellulose, bacterial cellulose is produced as a single polymer of pure cellulose and does not require laborious processing to remove lignin and hemicellulose. Despite progress in optimizing culture conditions to enhance bacterial cellulose production, less is known about the regulation of its synthesis. The regulatory molecule c-di-GMP binds the synthesis machinery needed for cellulose production. In *Escherichia coli*, c-di-GMP levels are repressed by the BolA protein. Two *bolA*-like genes have been identified in the *K. xylinus* genome. This study aims to investigate the role of these genes in modulating c-di-GMP levels and cellulose production by knocking down their expression using an inactive CRISPR system. Unlike the active CRISPR system, the inactive dead Cas9 (dCas9) system binds DNA without cutting, selectively inhibiting gene expression. We hypothesize that inhibiting the *bolA*-like genes in *K. xylinus* will increase c-di-GMP levels and enhance cellulose production. Results will characterize the function of these genes in cellulose synthesis and potentially lead to improved production yields.