

## **The role of the *Komagataeibacter xylinus* *bolA* homolog *kx1883* on cellulose production**

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Cellulose is the most abundant natural polymer on earth and is synthesized by both prokaryotes and eukaryotes. Among cellulose-producing bacteria, *Komagataeibacter xylinus* is the most studied because of its high bacterial cellulose production capacity. Bacterial cellulose has diverse applications in food, industrial processes, and biomedical fields (e.g., tissue engineering and drug delivery). Although plant and bacterial cellulose share the same molecular formula, bacterial cellulose is preferred because it is produced as a pure cellulose polymer. In contrast, plant biomass-derived cellulose is tightly linked with hemicellulose and lignin, making its separation laborious. The synthesis of bacterial cellulose depends on binding of the regulatory molecule cyclic-di-GMP (c-di-GMP). In many bacteria, c-di-GMP levels are negatively regulated by the protein BolA. However, the regulation of c-di-GMP remains unknown in acetic acid bacteria. In *E. coli*, BolA functions as a transcriptional regulator and *K. xylinus* encodes predicted *bolA* like gene *kx1883*. The use of a gene expression interference system called CRISPRi allows the knockdown of gene expression rather than deletion of gene function. Reducing *bolA* expression is expected to increase c-di-GMP levels and stimulate cellulose production. This research may help define previously unknown regulatory mechanism and support bioengineering of strains for industrial bacterial cellulose production.