Investigating the role of the *GXY_RS03165* histidine kinase on biofilm formation in *Komagataeibacter hansenii*

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Abstract

Komagataeibacter spp. are gram negative, acetic acid producing α-proteobacteria. These organisms thrive on senesced fruit and are model organisms for bacterial cellulose production. Cellulosic bacterial biofilms are produced in response to environmental triggers, where histidine kinases and response regulators propagate extracellular signals to elicit the intracellular response. The aim of this work was to investigate, in *K. hansenii,* the function of the *GXY_RS03165* gene which encodes a histidine kinase and putative GAF sensor protein. Preliminary bioinformatic analyses at the protein level identified a homolog with 100% identity in *K. xylinus,* annotated as a putative free methionine-R-sulfoxide reductase. The purpose of this study was to knockout the *GXY_RS03165* gene using overlap extension polymerase chain reaction (PCR). GXY_RS03165 function was disrupted through the insertion of an antibiotic resistance cassette. Colony PCR was performed on putative disruptants to confirm insertion of the resistance cassette. This work is the first step in discovering the mechanism by which the *GXY_RS03165* histidine kinase affects biofilm formation.

Introduction

Cellulose is one of the most abundant biopolymers found on Earth, as it is produced by plants, green algae, tunicates, and many bacterial species (Augimeri et al., 2015). Unlike plant cellulose, bacterial cellulose lacks hemicellulose, pectin and lignin, which provide it with a higher degree of crystallinity. It is due to these structural characteristics that bacterial cellulose application in industries has increased. Examples of its uses include tissue engineering, food products, and electronics (Fu et al., 2013; Lin et al, 2013).

Many bacteria produce cellulose as part of a biofilm that confers survival (Augimeri and Strap, 2015). Cellulosic biofilms are produced in response to environmental signals, where two-component signal transduction systems (2CSTS) propagate the extracellular signals to elicit an intracellular response (Prub, 2017). 2CSTSs involve a histidine kinase sensor protein and a response regulator, which employ a series of phosphotransfer mechanisms to elicit a response. Biofilm synthesis is also regulated by the second messenger c-di-GMP (Chen et al., 2016). Its levels are controlled by two classes of enzymes: diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), where DGCs catalyze its synthesis from GTP and PDEs hydrolyze it to linear pGpG and GMP.

Komagataeibacter hansenii is an acetic acid producing α-proteobacteria that produces a cellulosic biofilm at the air-liquid interface referred to as a pellicle (Augimeri and Strap, 2015). In *K. hansenii,* the *GXY_RS03165* gene encodes a histidine kinase and putative GAF sensor protein. GAF domains are named after the proteins where they are most commonly found in, including cGMP-specific phosphodiesterases, adenylyl cyclases, and FhlA. The purpose of this study was to investigate the function of the GXY_RS03165 histidine kinase and its effects on biofilm formation.

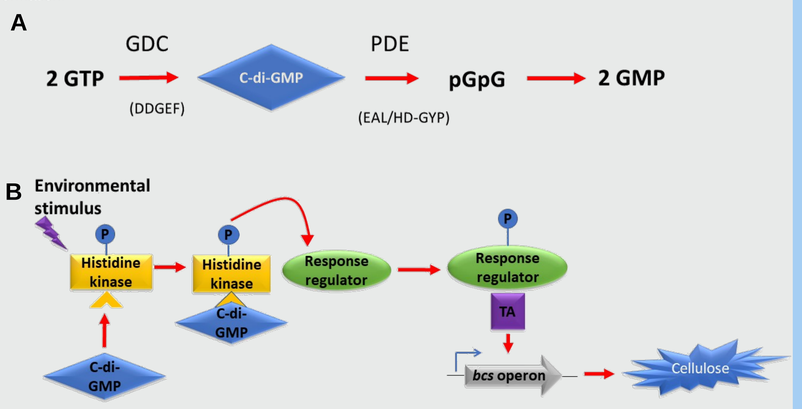

Figure 1. A) Synthesis and degradation of the second messenger, c-di-GMP. **B)** Bacterial cellulose production is activated through phosphorylation cascades. We propose that the second messenger c-di-GMP binds to a histidine kinase via its GAF domain, allowing for the phosphorylation of a response regulator and in turn the activation of a transcriptional activator (TA), leading to the upregulation of the *bcs* operon and the production of cellulose.

Methodology

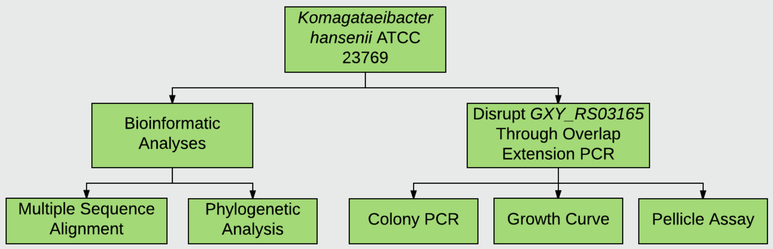

Figure 2. Scheme for investigating the role *GXY_RS03165* plays in growth and cellulose formation in *Komagataeibacter hansenii* ATCC23769.

Results

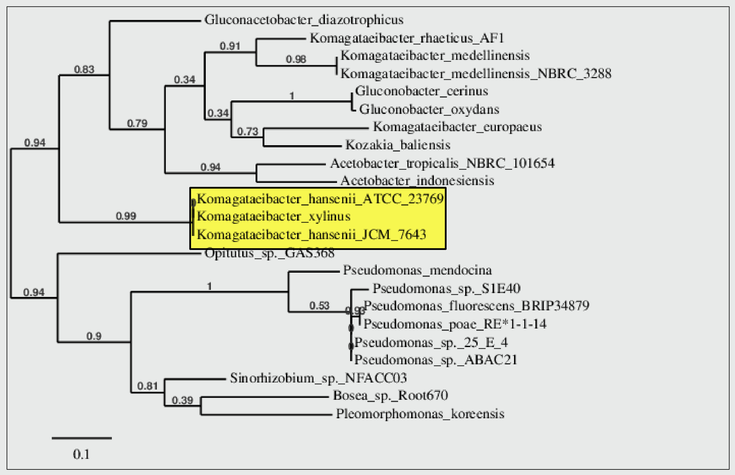

Figure 3. Preliminary bioinformatic analyses at the protein level identified GXY_RS03165 as a putative GAF sensor protein, as well as identified a homolog with 100% identity in *Komagataeibacter xylinus,* which encodes for a putative free methionine-R-sulfoxide reductase.

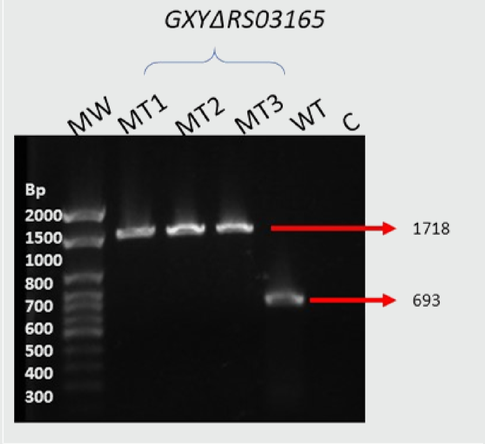

Figure 4. Colony PCR confirmed that the chloramphenicol resistance cassette was successfully inserted to disrupt *GXY_RS03165* in all three colonies tested.

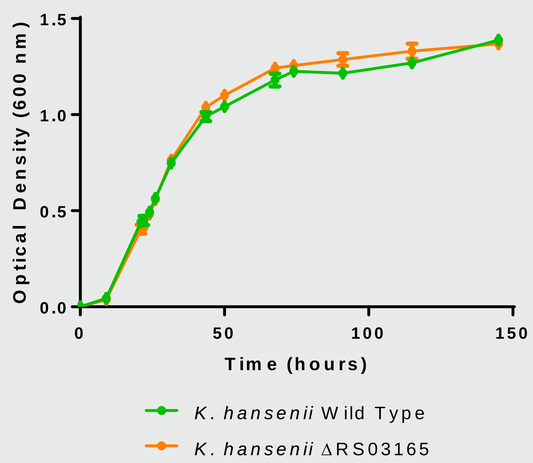

Figure 5. *ΔGXY_RS03165* does not significantly affect growth of *K. hansenii* (Student t-test, p≤0.05). Cultures were grown in Schramm-Hestrin medium with fructose as the carbon source under agitated conditions. Error bars represent standard error of the mean.

Results

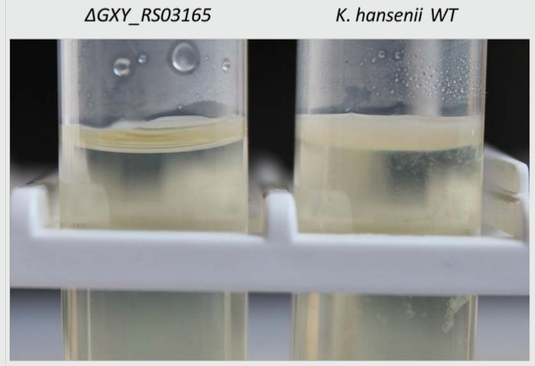

Figure 6. *ΔGXY_RS03165* abolishes pellicle production in *K. hansenii* ATCC 23769. Cultures were grown under static conditions in Schramm-Hestrin medium containing fructose as carbon source.

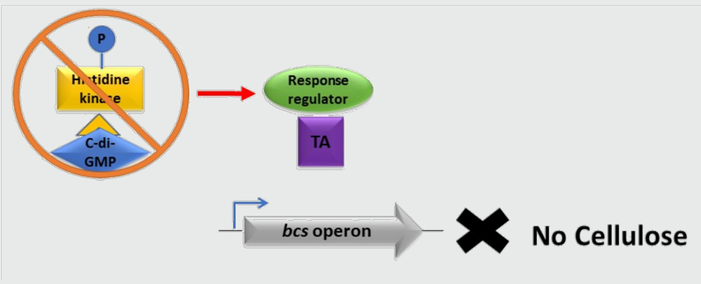

Figure 7. Proposed putative pathway for abolishment of cellulose production in *K. hansenii* ATCC 23769 due to *ΔGXY_RS03165*. The *GXY_RS03165* histidine kinase is essential for sensing the intracellular concentration of the second messenger, c-di-GMP. In the disruption mutant, c-di-GMP is not sensed, therefore the cognate response regulator is not phosphorylated and cellulose cannot be made.

Discussion/Conclusion

1. First study to investigate the involvement of histidine kinase in cellulosic biofilm formation of *Komagataeibacter spp.*
2. *GXY_RS03165* was successfully disrupted in *Komagataeibacter hansenii.*
3. *ΔGXY_RS03165* abolishes cellulose production in *K. hansenii* without affecting growth rate.
4. This is the second of two essential proteins for cellulosic biofilm formation in *Komagataeibacter hansenii* identified to date.

References

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Acknowledgements

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