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 semisweet fruit and are modeled organisms for bacterial cellulose production. Cellulotic bacterial infections are produced in response to environmental triggers, where histidine kinases and response regulators propagate extracellular signals to elicit an intracellular response. The aim of this work was to investigate 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 the study was to knock out 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, fungi, 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; Li 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 elicit a response. Biofilm synthesis is also regulated by the second messenger c-di-GMP (Chen et al., 2016). Its degradation is catalyzed by two classes of enzymes: diguanylate cyclases (DGCs) and phosphodiesterases (PDEs). The purpose of this study was to investigate the function of the GXY_RS03165 histidine kinase and its effects on biofilm formation in K. hansenii.

Komagataeibacter hansenii is an acetic acid producing α-proteobacteria that produces a cellulotic 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 were first identified: G-proteins, aRaf, and α-subunits of c-di-GMP.

Methods

GXY_RS03165 is an acetic acid producing α-proteobacteria that produces a cellulotic 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 were first identified: G-proteins, aRaf, and α-subunits of c-di-GMP.

A. Synthesis and degradation of the second messenger, c-di-GMP. B. Differential expression 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.

Results

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

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 chloromphenicol resistance cassette was successfully inserted to disrupt GXY_RS03165 in all three colonies tested.

Figure 5. GXY_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 agitation conditions. Error bars represent standard error of the mean.

Figure 6. GXY_RS03165 abolishes pellicle production in K. hansenii ATCC 23769. Cultures were grown under static conditions in Schramm-Hestrin medium containing fructose as the carbon source.

Discussion/Conclusion

1. First study to investigate the involvement of histidine kinase in cellulosic biofilm formation in Komagataeibacter spp.

2. GXY_RS03165 was successfully disrupted in Komagataeibacter hansenii.

3. GXY_RS03165 abolishes cellulose production in K. hansenii without affecting growth rate.

4. This is the second of two essential proteins for cellulose production in Komagataeibacter hansenii identified to date.

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References


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