Confirming the Necessity of *recA* for Natural Transformation in *Aeromonas salmonicida*

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Abstract

Aeromonas is a genus of bacteria whose members are ubiquitous in water. *Aeromonas* strains are known to be opportunistic pathogens and the causative agents of many gastrointestinal diseases. Natural transformation, the process of uptake and expression of free DNA, is a defining characteristic of the *Aeromonas* genus. The purpose of this project is to identify and confirm that the gene *recA* is involved in natural transformation. RecA is a protein that initiates recombination of broken DNA by encoding a DNA repair protein to ligate the ends back together. Preliminary studies in the lab suggest that this gene is important in transformation. *recA* has been removed from the genome using a technique called splice overlap extension PCR in combination with transformation. The resulting mutated strains of bacteria now have gentamicin resistance cassettes in place of *recA*. The mutants will then be scrutinized to determine the effects of the loss of the gene on natural transformation. *recA* will then be added back into the genome of *Aeromonas* and the antibiotic cassette will be removed to confirm the effects of *recA* on natural transformation.

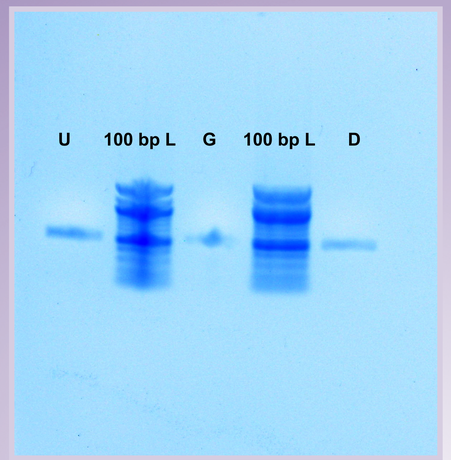

Figure 2. Gel image of upstream region (U), gentamicin cassette (G), and downstream region (D).

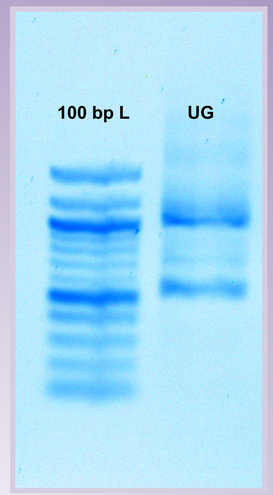

Figure 3. Gel image of upstream region and gentamicin cassette complex (UG).

Results and Discussion

The upstream and downstream fragments from the *recA* gene were successfully amplified, as well as the gentamicin cassette. However, creating the complete gene knockout has been proven to be more difficult and time consuming than expected. SOE PCR was used to anneal and amplify the upstream region and the gentamycin cassette, but through the use of gel electrophoresis it was determined that not all of the reactants annealed together. A DNA gel extraction was performed to isolate the complete upstream-gentamicin complex, but it continued to separate into its individual components. An attempt to make the entire knockout was tried many times, but the fragments continued to separate into individual pieces, no matter how many modifications were made to the SOE PCR procedure. Other options are continuing to be explored.

Conclusion

The lab is hopeful about the upcoming success of this experiment. Upon completing the knockout of *recA*, screening on Luria agar plates with gentamicin will be used to determine cultures of *A. salmonicida* that have successfully integrated the gentamicin cassette, and therefore the loss of *recA*, into their genome. UV radiation will then be used to screen for mutants that cannot repair their broken and UV-damaged DNA, indicating *recA* is lost. Next, the lab will confirm that natural transformation ability is also lost. The mutants will then be studied in relation to natural transformation. Finally, *recA* will be restored via a plasmid and colonies will regain their repair ability, confirming that *recA* has been successfully integrated back into the genome and is indeed involved in natural transformation.

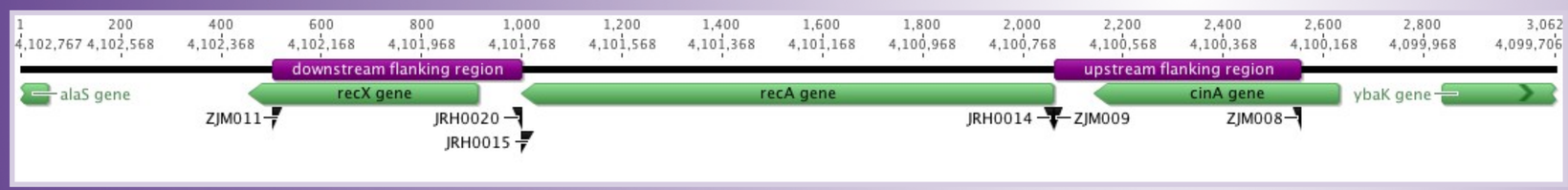

Figure 1. Map of *recA* gene region in *Aeromonas salmonicida*.

Introduction

Horizontal gene transfer is the movement of genetic material from one cell to another cell in a horizontal, rather than parental, fashion. A type of horizontal gene transfer is natural transformation, which is the uptake of free DNA by a cell. This may occur if a bacterium has lysed and died, leaving its genetic material in the extracellular environment. Another cell may use natural transformation to pick up the DNA and integrate it into its genome. This is relevant because some of the genetic material transferred could be genes for antibiotic resistance. *Aeromonas* species are opportunistic pathogens, resulting in gastroenteritis and skin infections. While relatively rare, these infections can be serious. Uptake and integration by *Aeromonas* of antibiotic resistance genes may cause species to become antibiotic resistant, resulting in limited options for physicians and healthcare professionals. Hence, the understanding and identification of the genes involved in natural transformation are vital in the progress of related research aiming to treat *Aeromonas* infections.

Materials and Methods

In this continuing experiment, the first major step was to create a knockout of the *recA* gene by replacing it with a gentamicin resistance cassette. The upstream and downstream regions of the gene of interest, *recA*, were separately amplified by PCR. The gentamicin resistance cassette to replace *recA* was also amplified. The upstream region and the gentamicin resistance cassette were then annealed together using splice overlap extension PCR (SOE PCR). The upstream/gentamicin complex was then annealed to the downstream region of *recA* to create a complete knockout of the gene. The final SOE PCR product will then be transformed into the wild-type and screened for mutants using gentamicin rich media. The mutants that successfully integrate the gentamicin cassette into their genome will then be studied to discover the effects of *recA* on natural transformation. Finally, the mutant will be complemented with the original *recA* gene, presumably reestablishing natural transformation ability. This will further confirm that *recA* is indeed necessary for natural transformation in *A. salmonicida*.

Citations

Huddleston, Jennifer, Joshua Brokaw, John Zak, and Randall Jeter. "Natural transformation as a mechanism of horizontal gene transfer among environmental Aeromonas species." *Systematic and Applied Microbiology* 36.4 (2013): 224-234.

Acknowledgements

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