Establishing *recA* as a Gene Involved in Natural Transformation Among Aeromonads

Zack Morgan and Dr. Jennifer Huddleston

Biology Department, Abilene Christian University, Abilene Texas 79699

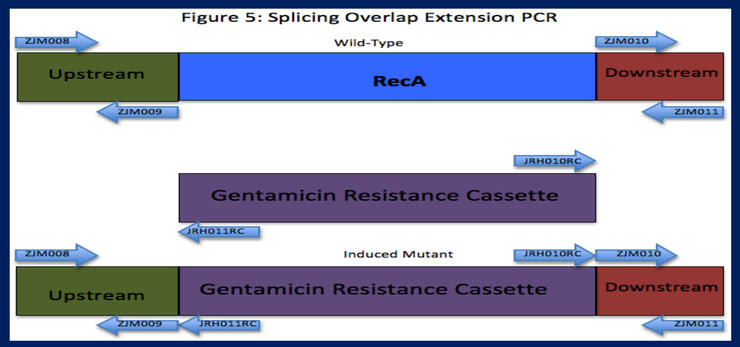
Abstract

Bacteria of the genus *Aeromonas* display the ability to naturally transform free-floating DNA from their environment. That is, these bacteria are able to incorporate extracellular DNA into their own genomes. While this process has been well documented, the source of these organism's capabilities to do so have not. This research project aims to establish the gene, *recA* as one of the principle genes allowing *Aeromonas* this ability. The gene *recA* encodes a DNA repair protein that is activated when DNA fragments are detected, and helps ligate the free ends back together. In the research reported here, *recA* was studied in an *Aeromonas* strain, *A. salmonicida.* It was confirmed to possess active RecA through UV radiation screening, amplified from the genome by PCR, sequenced, and aligned with known database sequences. This preliminary work sets the foundation to knock out the *recA* gene through mutating with a splicing overlap extension polymerase chain reaction (SOE-PCR) product, and to screen for a lost ability to transform DNA (in progress), thus providing evidence of RecA's role in natural transformation among aeromonads. This research is in conjunction with a larger lab group that, as a whole, is demonstrating the involvement of multiple genes with *Aeromonas'* ability to naturally transform DNA.

Introduction

Natural transformation is an ability of selected bacterial species to intake and incorporate free-floating DNA from their environments without external manipulation. Though a relatively rare ability, there are potential problems associated with natural transformation. Competence, or the readiness of a bacterium to transform DNA is often caused by situations or environmental circumstances that put selective pressures on the organism. Antibiotics used to eliminate bacterial infections have been proven to induce competence in some bacterial species. This could be problematic as it can lead to the spreading of antibiotic resistance genes among not only bacteria of the same species, but also bacteria of different species. Spreading antibiotic resistance has become a problem among medical practitioners and microbiologists alike, as research focusing on mitigating the effects of infections such as MRSA have become prevalent. This research is relevant in that it attempts to help explain the phenomena of natural transformation more expressly, and adds to the growing efforts to better understand and therefore better able to deal with the problems associated with natural transformation. The *recA* gene is a widely distributed gene that is involved in DNA repair frequently associated with UV degradation of DNA. This research aims to establish RecA's involvement in *Aeromanas'* ability to naturally transform environmental DNA.

Figure 5: Splicing Overlap Extension PCR



Biology Research

Acknowledgements

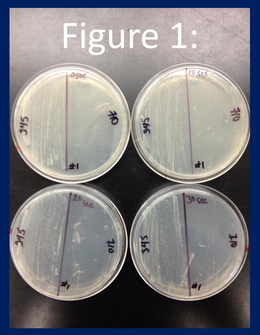
This project was supported by funds from an ACU Pursuit Grant (2012-2013) and an ACU Math-Science Award.

Materials and Methods

The first step in this experiment was to find an *Aeromonas* strain that possessed the *recA* gene. This was done by exposing multiple strains of this bacterium to varying intensities and durations of ultraviolet radiation. Figure 1 shows an experiment in which two *Aeromonas* strains, #345 and #710 were exposed to a low level UV light for 0s, 10s, 20s, and 30s as labeled. Figure 2 shows the results of an experiment in which the same two bacterial strains were exposed to a high-powered UV light for the same time increments. The continued proliferation of the bacterial colonies shows that the mutated DNA had been repaired through a mechanism that includes RecA activity. Then, using published *Aeromonas* genome sequences, seven primers were made to elongate the *recA* gene to be sequenced. The primers used can be found in Table 1. The exact protocol for the successful elongation PCR can be found in Table 2, and the gel can be found in Figure 3. After *recA* was successfully elongated, the DNA product was purified (Figure 4) and sent off for complete sequencing.

Table 1

	SEQUENCE (5'-3')	TM (*C)	PURPOSE
zjm001	TCCTGCTTGATTCGAATACG	53.9	PCR amplification of recA region
zjm002	CAAGAGCCAGCTCGAAC	56.1	Elongation of recA region for sequencing
zjm003	GAACAAGCTTCATGACGTTGG	54.2	Elongation of recA region for sequencing
zjm004	TAACGCGCTCAAGTTCTACG	55	Elongation of recA region for sequencing
zjm005	GCATGGATCAGAACAAACAGAAG	54.3	Elongation of recA region for sequencing
zjm006	GCGGCTTTGTCACCTATACC	55.9	Elongation of recA region for sequencing
zjm007	CGATCAGGGTCTTGAACACC	55.5	PCR amplification of recA region
zjm008	CTACCGCCGAATCCTGTAC	55	Initiate elongation for SOE PCR upstream
zjm009	GTTGCTGCTGCGTAACATGCCAATCTCCGCTGGAAAT	N/A	Create gentamicin overhang upstream
zjm010	TTAGGTGGCGGTACTTGGCAGTGAGGCCGGGCTAAC	N/A	Create gentamicin overhang downstream
zjm011	GGGCCTGATCCTGATTTAAACC	55.6	Initiate elongation for SOE PCR downstream
JRH010 (RC)	CCAAGTACCGCCACCTAA	N/A	Gentamicin upstream overhang
JRH011 (RC)	GTTGCTGCTGCGTAACAT	N/A	Gentamicin downstream overhang



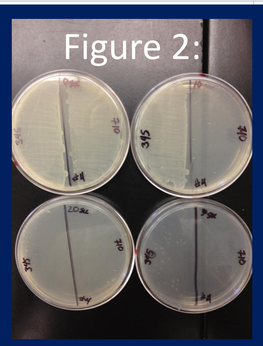
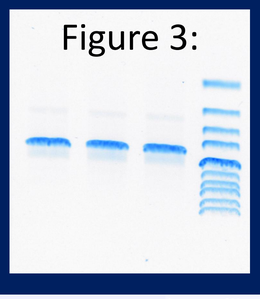
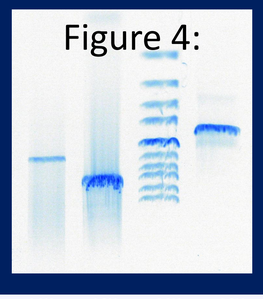


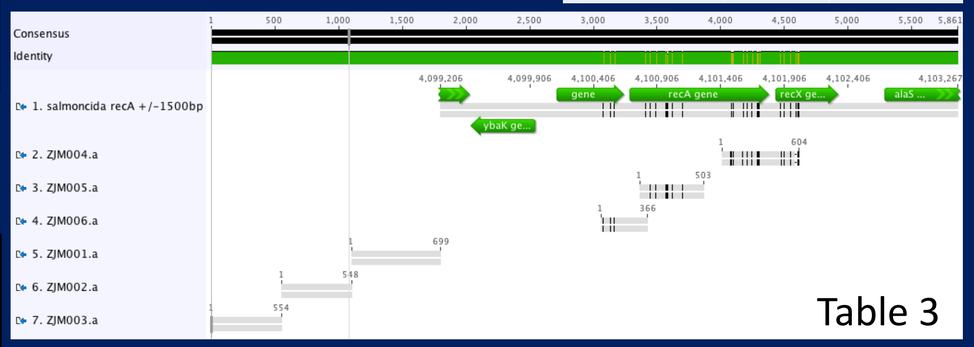
TABLE 2

Reagent (Concentration)	Volume (microliters)
dH_2O	158
AccuTaq Buffer	20
dNTP Mix (10mmol each)	4
ZJM001 & ZJM006 (10mmol each)	4 (each)
DMSO	4
Template DNA	4
AccuTaq Polymerase	2
Total: 200 microliters into 8 tubes of 25 microliters	
PCR Cycling Paramaters	

Step:	Temperature:	Time:
Initial Denaturation	94°C	1 min.
Cycles 1-29		
Denaturation	94°C	30 sec.
Annealing	51.4°C	30 sec.
Extension	72°C	3 min.
Final Extension	72°C	10 min.
Hold	12°C	∞







Results and Discussion

The low-level UV exposure inhibited the growth of strain #710 which had been proven to be a (random) *recA* mutant. This strain, however is not acceptable to be used as this experiment's *recA* mutant, as the mutation cannot be traced, and the experiment requires a site-directed *recA* mutation. In these UV exposure experiments, #345 serves as a control, as it is known to have an intact RecA protein. It can be observed, however that cell growth of #345 is inhibited at excessive exposure levels. This is likely due to a degradation of the RecA protein itself rendering it incapable of further DNA repair. An intact *recA* gene was screened for, because this experiment aims to demonstrate that inhibiting its activity depletes the bacterium's ability to naturally transform.

A summary of the sequencing data can be found in Table 3. This table outlines the percentage alignment of the found *recA* sequence with *A. hydrophila*, *Aeromonas veronii*, and the highest percentage match, *A.salmonicida*. This 98.2% pairwise identity shows that *recA* in the test strain #345 is effectively conserved from the published *A. salmoncida* genome.

The next step for the lab to begin is to replace the *recA* gene with a gene cassette for resistance to the antibiotic gentamicin. Seeing as how #345 has previously been proven to be susceptible to gentamicin, inserting this gene cassette will allow for the screening of a knocked-out *recA* gene. This gene knock-out will be accomplished through the means of Splicing Overlap Extension PCR (SOE PCR). This procedure is outlined in Figure 5. During this procedure, primers are designed that flank either end of the targeted gene to be replace on both the forward and compliment strand of DNA. These primers are then spliced to the trailing and leading end of the gentamicin cassette. Running a PCR with these primers will yield fragments A and C, with overhangs that correspond to the beginning and ending sequences of the gentamicin cassette. Once these overhangs have been produced, a subsequent PCR reaction will allow for the gentamicin cassette to bridge the gap between A and B via the overhangs effectively replacing *recA*'s place in the *Aeromonas* genome.

Once this has been accomplished successful replacement will be screened for by plating cultures onto plates containing gentamicin and indicated by successful cell growth. Additional confirmation of successful replacement will be demonstrated by the cell's lost ability to grow after being exposed to UV radiation as was previously demonstrated. Once the replacement of *recA* has been confirmed, the culture will be put through a natural transformation assay, which will produce results consistent for an inability to naturally transform. Finally, *recA* will be reintroduced to the cells via a plasmid, and it will be demonstrated through the natural transformation assay that the ability of natural transformation has been restored.

Conclusion

This research project is well under way to demonstrating the involvement of RecA in the natural transformation capabilities of aeromonads. Though still in progress, the foundational work has been done in this experiment to begin demonstrating the crucial role of RecA's DNA ligation abilities to naturally transform DNA. This research, once completed, will contribute to the public's knowledge and understanding of the phenomenon of natural transformation, and will thus increase modern understanding of the methods by which bacteria gain resistance to antibiotics- a growing threat to the well-being of the general population.

Works Cited

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