clpA/clpS genes and Natural Transformation in *Aeromonas*

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Abstract

Aeromonas are gram-negative bacteria that 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 newly discovered characteristic of the *Aeromonas* genus. A group of proteins, Clp proteins, are molecular chaperones that degrade damaged proteins in response to stress. The rarely investigated *clpA/clpS* gene encodes for a caseinolytic protease that preliminary studies by J.H. indicate might be necessary for natural transformation. *clpA/clpS* has been removed from the genome using a technique called splice overlap extension PCR, also known as SOE PCR. With the upstream region of the gene of interest designated "A", the gentamicin resistance cassette called "B", and the downstream region called "C", B was successfully annealed together, setting the stage for ABC to be annealed, resulting in the complete knockout. The mutated strains of bacteria will then be grown on a gentamicin rich media that will act a screen against strains that did not remove the genes. The mutants will then be scrutinized to determine the effects of the genes on natural transformation. The genes will then be added back into the genome of *Aeromonas* and the antibiotic cassette will be removed to confirm the effects of the genes of interest on natural transformation.

Introduction

Aeromonas bacteria are gram-negative rods that are found in aquatic environments. *Aeromonas* are known to cause opportunistic infections, and in some cases in the intestines of humans. *Aeromonas* is known as a competent strain of bacteria meaning that it is capable of uptaking and incorporating free DNA in a process called natural transformation. Natural transformation is a type of horizontal gene transfer that involves the uptake of foreign and free DNA from the environment. The receiving bacteria, in this research *Aeromonas*, take up the foreign DNA from the environment and the DNA incorporates itself into the receiving bacteria's genome. This case of transformation is called natural because *Aeromonas* and other bacteria like it naturally have the genes necessary for this process to occur in their genomes. The goal of this research is to discover which genes are necessary for the process of natural transformation to occur in *Aeromonas*. My research focused on the ClpA/ClpS proteins. Clp proteins are molecular chaperones that degrade damaged proteins during any stress response. More specifically *clpA/clpS*, the genes that are of the main focus of my research, encode a caseinolytic protease. These genes have rarely been studied and will provide incredible insight into the function of this gene in the process of natural transformation.

Acknowledgements

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Materials and Methods

Preliminary research involved the screening of *Aeromonas* mutant strains for the confirmation of the presence of *clpA/clpS*. Strain 345 of *Aeromonas* showed to have the wild-type *clpA/clpS* gene. After strain 345 was isolated, primers were designed using Geneious, a program used to investigate genomes. Primers were designed to amplify the *clpA/clpS* sequences using PCR. *ClpA/clpS* was successfully amplified and sent to another lab to be sequenced. The sequence of *clpA/clpS* was received and primers necessary for SOE PCR, or splice overlap extension PCR, were designed. First, the upstream and downstream flanking regions and a gentamicin cassette marker were amplified for SOE PCR. Using AccuTaq polymerase various PCRs were run to find the optimum temperature and extension time needed to correctly amplify the flanking regions and cassette. 1 cycle of 95C for 3min, 30 cycles of 95C for 30sec, 56C for 30sec, and 72C for 1min, then 1 cycle of 72C for 10min.

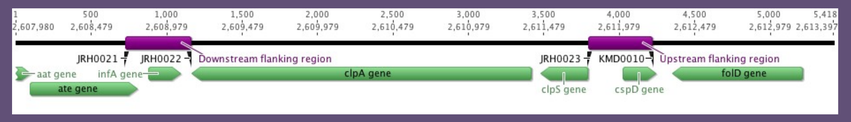

Figure 1. Map of *clpA/clpS* gene showing upstream and downstream flanking regions.

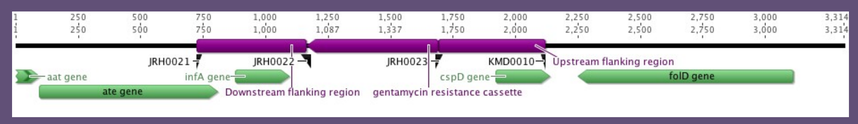

Figure 2. Map of *clpA/clpS* gene replaced by the gentamicin cassette marker.

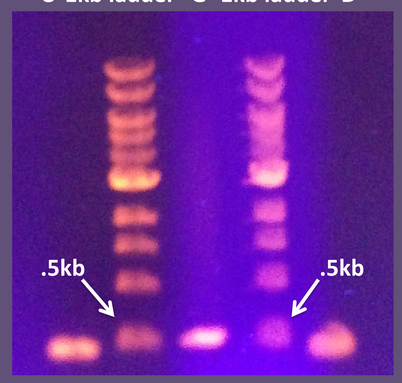

Figure 2. Photo of ethidium bromide 1% gel electrophoresis showing successful amplification of the upstream region (U) , gentamicin cassette marker (G), and downstream region (D) of *clpA/clpS*.

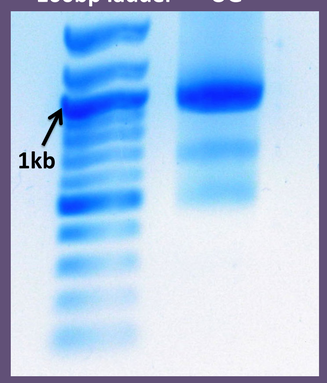

Figure 3. Photo of ethidium bromide 1% gel electrophoresis showing successful annealing of the upstream region and gentamicin cassette marker (UG) of *clpA/clpS* by SOE PCR.

Materials and Methods cont.

The amplification reactions contained 10x AccuTaq reaction buffer, 2.5mM of each dNTP, 0.5microliter of AccuTaq DNA polymerase, 0.1 micromolar of each primer, and 0.5microliters of template DNA for a final reaction of 50microliters. Once the products for SOE PCR were successfully amplified and confirmed by gel electrophoresis, AccuTaq polymerase was used in various PCRs to find the optimum temperature and extension time needed to correctly anneal the upstream flanking region and gentamicin cassette marker together. 1 cycle of 95C for 3min, 1 cycle of 55C for 1min, 1 cycle of 72C for 6min, then the primers designed for SOE PCR were added to the PCR reaction, then 30 cycles of 95C for 30sec, 55C for 30sec, and 72C for 2min , then 1 cycle of 72C for 10min. Amplification reactions contained 10x AccuTaq reaction buffer, 2.5mM of each dNTP, 0.5microliter of AccuTaq DNA polymerase, 0.1 micromolar of each primer, and 1.0microliter of template DNA for a final reaction of 50microliters. A gel electrophoresis was used to confirm the successful annealing of the upstream flanking region and the gentamicin cassette.

Results and Discussion

Preliminary tests confirmed that wild type *clpA/clpS* that was amplified and sequenced was found to be from *Aeromonas salmonicida*. The preliminary products needed for SOE PCR, the gentamicin cassette marker and upstream/downstream flanking regions, were successfully amplified in *clpA, clpS, and clpA/clpS.* The correct product size of the amplified DNA was confirmed by gel electrophoresis. The upstream flanking region and gentamicin cassette marker were successfully annealed in *clpA, clpS*, and *clpA/clpS* as is confirmed by gel electrophoresis.

Conclusion

Now that the upstream flanking region and gentamicin cassette are successfully annealed together, the downstream flanking region must be annealed to the DNA fragment. This will be done by SOE PCR. The DNA fragment made will be introduced to *Aeromonas* cells to be recombined into the it's genome. The mutated strains of bacteria will then be grown on a gentamicin rich media that will act a screen for strains that did replace the *clpA/clpS* genes. The mutants will then be scrutinized to determine the effects of *clpA/clpS* on natural transformation. The *clpA/clpS* genes will be added back into the genome of *Aeromonas* by use of a complementation vector to confirm the effects of the *clpA/clpS* on natural transformation.

Citations

1. Huddleston, J. R., Brokaw, J. M., Zak, J. C., & Jeter, R. M. (2013). Natural Transformation as a Mechanism of Horizontal Gene Transfer Among Environmental *Aeromonas* species. Systematic and Applied Microbiology, 36(4), 224–234.
2. Parker, Jennifer L., Shaw, Jonathan G. (2011). *Aeromonas* spp. Clinical Microbiology and Disease. Journal of Infection. 6,110-111.