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 also 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 c*lpA/clpS* gene encodes for a caseinolytic protease that preliminary studies by J.H. indicate might be necessary for natural transformation. For this current research, mutant *Aeromonas* strain 345 was screened for the presence of *ClpA/ClpS*. Strain 345's DNA was successfully isolated and amplified by PCR. The amplified genetic material was then sequenced. The sequence verified that environmental isolate strain 345 was *A. salmonicida*. The progress made so far is the groundwork for the next step of research that is to knock out and replace the *clpA/clpS* genes using a technique called splicing overlap extension PCR, also known as SOE PCR. A cassette of genes will be inserted in the transformed bacteria to act as a marker for the success of the gene replacement. The mutants will then be scrutinized to determine the effects of the lack of *clpA/clpS* 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

Many strains of *Aeromonas* have been cultured and mutated by Dr. Huddleston. At the very beginning of research, the team made a trip to Texas Tech University where 400 strains of *Aeromonas* were subcultured. Two screening protocols were designed to test the presence of the *clpA/clpS* gene in *Aeromonas* mutant strains 771 and control strains 695 and 345. The first screening protocol involved the preparation of LB+gentamicin and LB+ampicillin plates. Mutant and control strains were streaked and incubated at 30C for 24 hours. The second protocol designed for confirmation of *clpA/clpS* mutants involves the preparation of casein agar plates.

Identification	Primer sequence	T_m	Purpose
KMD0001	CACTATTCTCGAAACCCTGC	53.2 ºC	amplifying clpA/clpS gene
KMD0002	ATGAGCCACGATGCCATG	55.7 ºC	sequencing clpA/clpS gene
KMD0003	CGTTGCCAAGATTGCCCGT	52.2 ºC	sequencing clpA/clpS gene
KMD0004	TCATCAAGCCGTTGCTCT	54.2 ºC	sequencing clpA/clpS gene
KMD0005	ATTGAGAGCTTTGCCACC	53.0 ºC	sequencing clpA/clpS gene
KMD0006	AACGAGGCTTTCAAACTG	50.5 ºC	sequencing clpA/clpS gene
KMD0007	GGTTGCCATACATCTACATCC	53.1 ºC	sequencing clpA/clpS gene
KMD0008	TCTGGAACAACAGGTGCG	55.3 ºC	amplifying clpA/clpS gene
KMD0009	CGCTTCACTCAGGATCTG	52.7 ºC	amplifying clpA/clpS gene

Figure 1. Primers designed for amplification of the *clpA/clpS* gene found in *Aeromonas*.

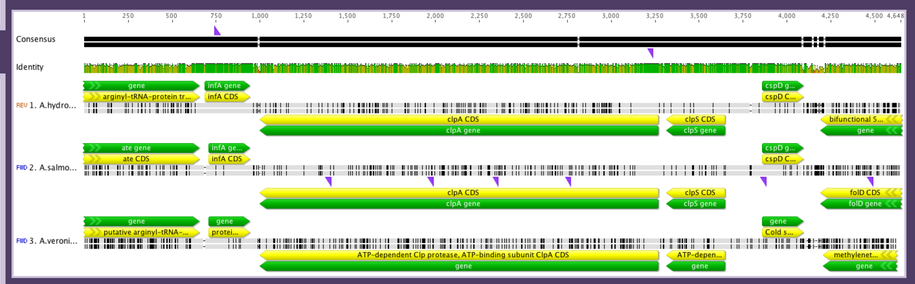

Figure 2. Primer map of designed primers for the amplification of the *clpA/clpS* gene found in *Aeromonas*. Species used *Aeromonas hydrophila, Aeromonas salmonicida, Aeromonas veronii*.

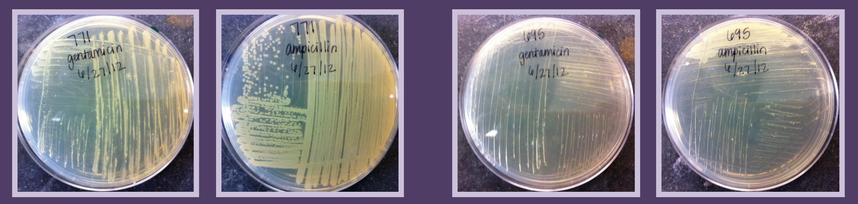

Figure 3. Photos of incubated LB+ampicillin/LB+gentamicin plates of strains 695 and 771 for proof of absence of the *clpA/clpS* gene.

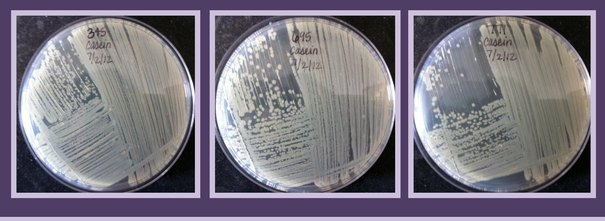

Figure 4. Photos of incubated casein plates streaked with strains 695, 345 and 771 used for confirmation of absence of the *clpA/clpS* gene.

Materials and Methods cont.

Strains 771, 695, and 345 were streaked on casein agar plates and incubated for 24 hours. To begin research the DNA of strain 345 of *Aeromonas* had to be isolated. This strain of *Aeromonas* has the wild-type *clpA/clpS* gene and the other genes of interest present in its genome. After strain 345 DNA was isolated, primers were designed using Geneious, a program used to investigate genomes. Nine primers were designed based on *A. salmonicida, A. hydrophila,* and *A.veronii* to amplify the *clpA/clpS* sequences. Using NEB Taq polymerase various PCRs were run to find the optimum temperature and extension time needed to correctly amplify the *clpA/clpS* gene on *Aeromonas*. 1 cycle of 94C for 1 min, 30 cycles of 94C for 30sec, 55C for 30sec, 72C for 5min, and 1 cycle of 72C for 10min. Amplification reactions contained 1x Accutaq reaction buffer, 2.5mM of each dNTP, 0.5 microliter of Accutaq DNA polymerase, 0.1micromolar of each primer, and 40 nanograms of template DNA in a a final reaction volume of 50 microliters. Finally, the genes were correctly amplified and were sent off to another lab to be sequenced .

Results and Discussion

Strain 771 indicated growth on both LB+gentamicin and LB+ampicillin plates proving the absence of the clpA/clpS genes. Strain 695 showed no growth on the LB+gentamicin plate and a small amount of growth on the LB+ampicillin plate (*Aeromonas* bacteria are borderline ampicillin resistant). All strains showed zones of casein lysis around isolated colonies in the casein plate screening procedure. The sequences returned showed that the strain closest to what was isolated was *Aeromonas salmonicida*. The sequences of DNA acquired from the primers designed showed alignment of the sequenced portions of DNA and *Aeromonas salmonicida* acquired through PCR.

Conclusion

Now that the genes of interest are amplified, an antibiotic cassette of gentamicin must now also be amplified using a plasmid. Gentamicin will be used as a marker for the absence of the genes of interest. The *clpA/clpS* genes will then be removed from the genome of the strain 345 using a technique called splicing overlap extension PCR, also known as SOE PCR. In the place of *clpA/clpS*, the antibiotic cassette of gentamicin will be placed as a marker. The mutated strains of bacteria will then be grown on a gentamicin rich media that will act a screen for strains that did not remove the *clpA/clpS* genes. The prototrophs will then be scrutinized to determine the affects of *clpA/clpS* on natural transformation. The *clpA/clpS* genes will be added back into the genome of *Aeromonas* and the antibiotic cassette will be removed to confirm the affects of the genes of interest on natural transformation.

Citations

1. J. R. Huddleston. 2008. Natural transformation in Aeromonas species. Texas Tech
University:.5,75-76.
2. Parker, Jennifer L., Shaw, Jonathan G., 2011.Aeromonas spp. clinical microbiology and disease.
Journal of Infect. 62:110-111.