Investigating the roles of *clp* genes in the natural transformation ability of *Aeromonas salmonicida*

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Abstract

Organisms of the genus *Aeromonas* are ubiquitous in water and can be identified as Gram negative, rod-shaped, facultatively anaerobic bacteria. These bacteria are opportunistic extraintestinal and intraintestinal pathogens that cause disease year-round and with varying severity. *Aeromonas salmonicida* is a naturally transformable species. Natural transformation is a six-part process that results in a bacterium incorporating free DNA into its genome. It is possible that aeromonads can become antibiotic resistant through this mechanism. The proteolytic molecular chaperones ClpA and ClpS have been identified as affecting the natural transformation process of the genus *Aeromonas* organisms. In this investigation, the effect of ClpA and ClpS on the natural transformation ability of *Aeromonas* species were studied separately and in conjunction with each other by creating three different mutant fragments for use in gene replacement. The three mutant fragments constructed replaced the genes of interest - *clpA*, *clpS*, or *clpA*/*clpS* - with a gentamycin resistance cassette. These fragments were assembled through PCR and SOE-PCR. Having constructed the *clpA* mutant fragment and having made significant progress on the *clpS* mutant fragment and the *clpA*/*clpS* double mutant fragment, the project is now focusing on gene replacement in the wild-type *Aeromonas* strain with the intention of making a comparison between the wild-type and mutant strains which will enable the determination of the effect of these Clp proteins on the natural transformation ability of *Aeromonas salmonicida*.

Introduction

Bacteria in the genus *Aeromonas* are ubiquitous in aquatic environments. These mesophilic bacteria have been found in domestic sewage, rivers receiving sewage discharges, clean rivers, lakes and storage reservoirs, as well as drinking water in distribution systems (1). Aeromonads are known to cause a variety of diseases in humans. These are pathogens that can infect healthy humans of all ages with varying degrees of severity through a number of different modes of infection (2, 3, 4). In addition, Sakai (5) and Huddleston et al. (6) reported that *Aeromonas salmonicida* is capable of achieving competence and undergoing natural transformation. In this process, a bacterial cell obtains free DNA from the environment to use for survival in its environment. The cell requires the participation of certain cellular functions that are provided by genes located throughout the bacterial chromosome and that may only be expressed under particular environmental conditions (7). The competence-inducing conditions for *Aeromonas* are known to include nutrient-limiting conditions and stationary phase of growth (6). Transformation by free DNA is more likely to occur in habitats with high bacterial concentrations. This environment exists in the intestines of insects, worms, and warm-blooded animals including the human stomach and intestines (7). The presence of antibiotics is stressful enough to induce competence in bacteria. Antibiotics create an environment in which antibiotic-resistant populations are selected. These antibiotic-resistant populations then expand clonally and through horizontal gene transfer (8). Since *Aeromonas* can survive in the intestines and causes gastrointestinal infections, the prospect of this bacteria becoming antibiotic-resistant increases, and therefore, is more difficult to treat. If the processes of horizontal gene transfer were understood, the acquiring of antibiotic resistance could be averted along with the loss of human lives. Clp proteins refold, remodel and degrade proteins damaged under stress conditions (9, 10). It is reasonable to conclude that there could be an increase in the presence or activity of ClpA at the time of natural transformation. To test for the importance of ClpA in the natural transformation ability of *Aeromonas salmonicida*, a mutant fragment containing a gentamycin resistance cassette was constructed to replace *clpA* in the wild-type *Aeromonas* genome.

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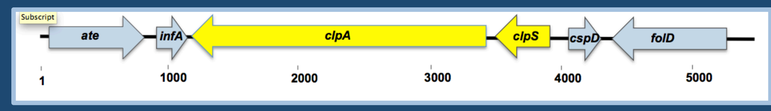
Fig. 1. Map of the *clp* region in the *Aeromonas* wild-type genome.



Materials and Methods

Mutant Fragment Construction. Using the previously extracted DNA from *Aeromonas salmonicida* strain #92, approximately 500 base pairs from the downstream and upstream *clpA* region (Fig.1) were amplified through Polymerase Chain Reaction (PCR) using the designed primers. The gentamycin resistance cassette (Gm^R) was amplified through PCR from *Aeromonas* strain #771 which has been engineered to contain the gene for gentamycin resistance (gm^R). The PCR products were purified using the Zymo Research DNA Clean & Concentrator™-25 kit (Irvine, CA). Splicing overlap extension polymerase chain reaction (SOE-PCR) (11) was used to combine the three amplified pieces. First, the gentamycin resistance cassette (gm^R) and the upstream *clpA* region were combined using this method. Then, the downstream *clpA* region was attached to this product using SOE-PCR (Fig. 2) followed by a gel extraction to ensure the isolation of the pure mutant fragment.

Fig. 2. SOE-PCR Scheme: Splicing Overlap Extension Polymerase Chain Reaction

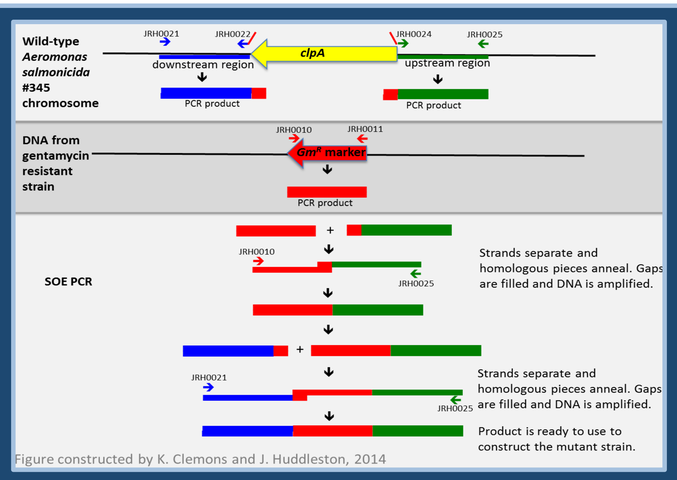


Figure constructed by K. Clemons and J. Huddleston, 2014

Fig. 3. Mutant fragment construction. Left gel: PCR amplification of *clpA* flanking regions and the gm^R cassette, **Middle gel:** SOE-PCR combination and amplification of upstream *clpA* region/gm^R, **Right gel:** PCR amplification of the *clpA* mutant fragment.

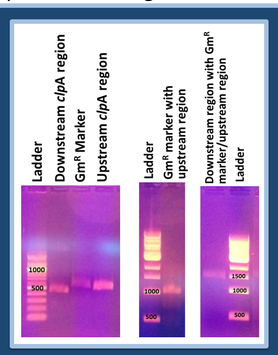
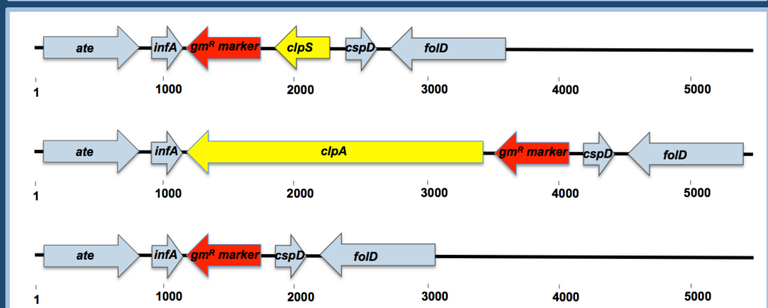


Fig. 4. Gene map of completed and confirmed *clpA* mutant fragment.

Results

As can be seen in Fig. 3 and Fig. 4, the *clpA* mutant fragment was successfully constructed. Constructing the *clpA* mutant fragment is the first step to achieving an understanding of the role of ClpA in the natural transformation ability of *Aeromonas salmonicida*. Now that this has been accomplished, gene replacement of the *Aeromonas* wild-type is possible and is currently in progress. The gene replacement will allow for a comparison of the wild-type and mutant bacteria which will enable determination of the extent of involvement of ClpA in the natural transformation ability of *Aeromonas salmonicida*.

Fig. 5. Maps of genes replaced with gm^R cassette in the genome. Top: *clpA* replaced, **Middle:** *clpS* replaced, **Bottom:** *clpA* and *clpS* both replaced



Discussion and Conclusion

The downstream and upstream *clpA* regions of the mutant fragment act as recognition regions for the *clpA* site in the genome. The homologous regions should anneal to each other, causing the mutant fragment to replace *clpA* in the wild-type *Aeromonas* genome. The Gm^R serves as a selectable marker. The study of natural transformation in the genus *Aeromonas* is only beginning. Huddleston (12) implicated four genes in the ability of aeromonads to naturally transform. Interestingly, ClpA has never been implicated in natural transformation in any other strain. However, ClpCP in Streptococcus (13) and ClpC in Campylobacter (14) has been shown to play a role in transformation. The construction of this *clpA* mutant fragment has paved the way for projects to come. In addition to launching the creation of the *clpA Aeromonas* mutant, this method will be used to construct mutant fragments for the study of *clpS* and *clpA/clpS* in the natural transformation ability of *Aeromonas* as well as other genes suspected of involvement in its natural transformation ability (Fig. 5).

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