Creating a Differential Medium for an Aeromonas salmonicida clp Mutant that is Deficient in Transformation

Jacob Woods and Dr. Jennifer Huddleston
Biology Department, Abilene Christian University, Abilene Texas 79699

Biology Research

Abstract

Natural transformation is the process by which bacteria directly uptake, incorporate, and express free genetic material from the environment as a result of the bacteria being in a state of competence. The Huddleston lab previously isolated an *Aeromonas salmonicida* strain with a mutation in the *clp* genes that cannot be naturally transformed. The *clp* genes in bacteria normally function as a chaperone protein which degrades damaged or misfolded proteins created in response to environmental stresses. This particular set of proteins has not been associated with natural transformation in any other genus of bacteria. The first objective of this study was to develop a growth medium that can differentiate *A. salmonicida* strains with the wild type *clp* genes from those with mutant *clp* genes. The development of this differential medium allows for future investigations by other members of the Huddleston research team, including screening for mutations with site-directed mutations of the *clpA* and *clpS* genes. The second objective of this study was to perform growth curves to compare the growth patterns of the wild type and the mutant strains in different types of media and in different growth conditions. The results of this research allow for further investigations into other functions of the *clp* genes.

Introduction

Aeromonas salmonicida are gram-negative rods found in aquatic environments and have been found to cause opportunistic infections in the intestines of humans. The genus *Aeromonas* possesses a characteristic known as natural transformation, which is the ability to uptake and incorporate plasmids into the bacterium's genome. Natural transformation, a type of horizontal gene transfer, is achieved when the aeromonad is subjected to environmental stress, and thus is induced to competence. This process is referred to as "natural," since this genus and related genera possess the genes needed for the uptake and incorporation of free DNA into the bacterial genome. Recently, a strain of *A. salmonicida* was constructed with a mutation in the *clp* genes that is not able to be naturally transformed. Normally, *clp* genes function as a chaperone protein which degrades damaged proteins and also as a caseinolytic protease. These proteins have not been associated with natural transformation in any other bacterial genus. The goal of this research was to develop a differential medium to distinguish between isolates with wild type *clp* genes and those with the mutant *clp* genes. Growth studies were then performed to compare the growth patterns of the wild type and mutant strains in various media and growth conditions. This research focused on the *ClpA/ClpS* proteins in the wild type and mutant strains, which might be associated with natural transformation in this species. The results of this study allow for future investigations into other functions of the *clp* genes, including screening for mutations with site-directed mutations of the *clpA* and *clpS* genes.

Acknowledgements

This project was supported by funds from an ACU Pursuit Grant (2013-2014).

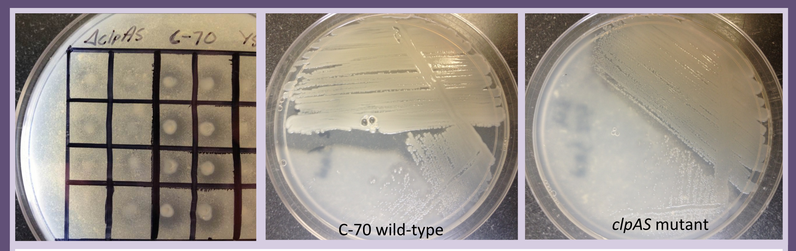

Figure 1. Growth of wild-type C-70 and *clpAS* mutant on 0.2× casein medium. Left: Spots tests of strains. **Middle**: 0.2× casein agar plate streaked with C-70 wild type**. Right:** 0.2× casein agar plate streaked with *clpAS* mutant**.**

Materials and Methods

Differential medium development. In order to develop a differential casein medium to distinguish between the growth of the wild-type and the *clp* mutant, we referred to a simple casein agar medium protocol (1×) of 10.0 g of agar and 50.0 g skim milk powder per liter, 0.5× (25 g milk per liter), and 0.20× (10 g milk per liter). The media were then autoclaved at 121°C for 20 minutes. The *A. salmonicida* wild-type strain (C-70) and the *clp* mutant strain (Δ*clpAS*) (which was derived from C-70) were streaked onto 1× casein agar plates, incubated for 24 hours at 30°C, and observed to detect a different appearance in growth between strains. However, little difference in appearance between the strains was observed, possibly due to the rapid rate of growth of each strain. In order to adjust for the discrepancies in growth, isolated colonies from each *Aeromonas* strain stock plate were inoculated onto 0.5× and 0.2× casein agar plates, incubated at room temperature (~25°C) for 24 hours, and observed to detect a difference in growth.

Growth curves. Growth curves were performed by inoculating the wild-type and mutant in three types of media: Tryptic Soy Broth (TSB), 15% Nutrient Broth (NB), and milk. Starter cultures were inoculated into TSB and incubated overnight. The grown cultures were then diluted in fresh medium to an A_{600} of 0.180 and then 1 ml was inoculated into 250 ml of fresh medium in a flask. Every hour for 10 hours, 4 mL samples were taken from each inoculated flask and the absorbance at 600 nm was measured with a spectrophotometer.

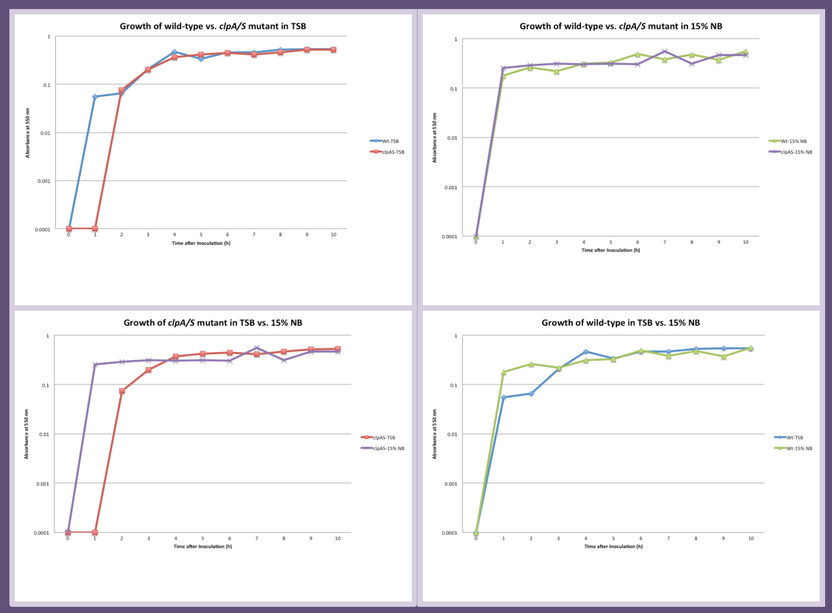

Figure 2. Growth curves illustrating the comparison of the wild-type and mutant strains in TSB and 15% nutrient broth at an absorbance of 600 nm over a 10- h period.

Results and Discussion

The best differentiation in growth between the *clpAS* mutant strain and the C-70 wild-type strain was indicated on the 0.2× Casein Agar. As shown in Figure 1, the cleared zone around the bacterial colonies is greater around the wild type than the mutant. This indicates a greater degradation of casein by the caseinolytic proteases of the C-70, so this medium can be used to differentiate between the wild-type and the mutant strains. The casein degradation phenotype of the *Clp* proteins is quickly assayable on this medium. The appearance of these inoculated plates will serve as an indicator of the activity of the *Clp* proteins, which will aid in the understanding of their role in natural transformation in upcoming studies.

The growth curves need to be replicated in order to make any definite conclusions, since there was only time to do one trial run. The four growth curve graphs (Figure 2) that showed a difference in growth comparing the wild type and mutant strains are the 15% NB comparing the wild-type and mutant; the TSB comparing the wild type and mutant; the growth of the mutant in TSB and 15% NB; the growth of the wild-type in TSB and 15% NB. We decided to use 15% NB for growth, since this is the favored growth condition in which bacterial cells become competent. Growth of the wild-type was faster in the TSB compared to the growth of the mutant in TSB. It appears as if the mutant grew faster in the 15% NB compared to the mutant growth in TSB. However, the growth of the wild-type and mutant in 15% NB were approximately the same, and no valid reasons are currently known to explain this event. It also appears as if the wild-type grew faster in the 15% NB compared to the wild-type growth in the TSB.

Conclusion

Now that a differential casein medium has been developed to differentiate the growth between the C-70 wild-type strain and the mutant *clpAS* strain, we can use this medium in future studies to investigate whether the *clp* genes are involved in natural transformation of *Aeromonas.* We will carry out several more repeats of the growth curve experiment in order to establish a reliable set of growth curve data. This will allow us to draw further conclusions on the reasons for a difference in appearance of growth between the mutant and wild-type *Aeromonas* strains.

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.