The *tapY1* gene and natural transformation in *Aeromonas*

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Abstract

Horizontal gene transfer is an important field of study as it refers to the transmission of genetic information between two different organisms by means other than traditional reproduction. Transformation is a type of horizontal gene transfer in which a cell is capable to uptake foreign DNA found in its environment and incorporate it into its own genome for expression. Some species of bacteria, such as *Aeromonas* are capable of doing this naturally, which accounts for both gaining and losing of characteristics, including antibiotic resistance. The *tapY1* gene found naturally in *Aeromonas* encodes a type IV pili biogenesis protein which allows the cell to be able to adhere to neighboring cells and surfaces. This gene is hypothesized to play a role in natural transformation by giving the cell the ability to bind to DNA. The *tapY1* gene was targeted and amplified using primers and polymerase chain reaction to allow us to target the region the *tapY1* gene occupies for replacement. Removal of the *tapY1* gene and insertion of the gentamycin resistance gene will result to the loss of the type IV pili protein and its ability to adhere to surfaces but gain expression of gentamicin resistance. The *tapY1* mutant constructed for study can then be tested for a change in natural transformation ability.

Introduction

The genus *Aeromonas* is comprised of gram negative rod-shaped bacteria that inhabit aquatic environments and are widely distributed in freshwater, estuarine, and marine waters (1,2). *Aeromonas* has been linked to causing a wide spectrum of diseases among both warm- and cold-blooded animals, including fish, amphibians, mammals, reptiles, and humans (3,4). In humans alone, *Aeromonas* produces important human pathogens causing primary and secondary septicemia in immunocompromised persons, serious wound infections in healthy individuals, and have also been attributed to a number of other illnesses such as meningitis, eye infections and a recent disease of interest, gastroenteritis (5). Natural transformation is a type of horizontal gene transfer in which cells can freely uptake DNA from different species within its environment rather than inheriting traits vertically from parent to offspring. Bacteria capable of performing natural transformation can incorporate new DNA into its genome and can have profound effects on the cell in respect to the bacteria's abilities and characteristics. So far, natural transformation in *Aeromonas* has not been widely studied and does not have much research behind it, but determining it's capability to perform natural transformation can have major future implications on antibiotic resistance and drug susceptibility. This research focuses on the *tapY1* gene sequence of *Aeromonas salmonicida* and its effects on natural transformation. The *tapY1* gene encodes a type IV pili biogenesis protein which accounts for adherence factors of the cell and is also hypothesized to play a role in natural transformation by giving *Aeromonas* the ability to bind to DNA. To study the effect of the *tapY1* gene, the *tapY1* gene will be isolated from the bacterial genome and will be replaced with a gentamycin cassette. The resulting strain will no longer have the *tapY1* gene contained in its genome and the mutant *Aeromonas salmonicida* strain can then be studied in its natural transformation abilities in the absence of the *tapY1* gene.

Acknowledgements

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Materials and Methods

Mutant Construction. A Splice Overlap Extension (SOE) PCR strategy was designed in order to construct a DNA fragment to transform into wild-type *A. salmonicida* in order to replace the *tapY1* gene with a gentamycin resistance cassette. K. Goodwin (former Huddleston lab member) designed primers to amplify 500 bp of flanking sequence upstream and downstream of the the *tapY1* gene. KLG001 and KLG002 were used to amplify the upstream flanking region "A" and KLG003 and KLG004 for the downstream flanking region "C" (Figure 1). The gentamycin resistance cassette ('B") from pTn*Mod*-OGm (6) was amplified with primers JRH0010 and JRH0011. SOE PCR was performed to combine product "A" and 'B" together using primers KLG001 and JRH0011 to form a newly constructed product "AB".

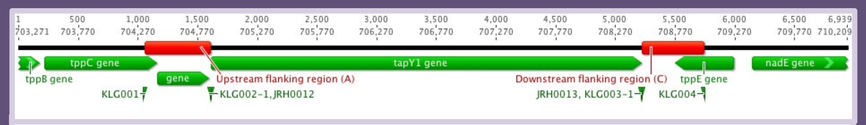

Figure 1. Map of primers for the amplification of *tapY1* gene in the wild type *A. salmonicida* as well as the upstream and downstream flanking regions.

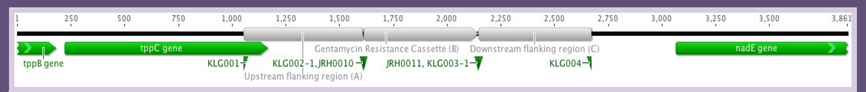

Figure 2. Map of *A. salmonicida tapY1* gene replaced with the getamycin resistance casette.

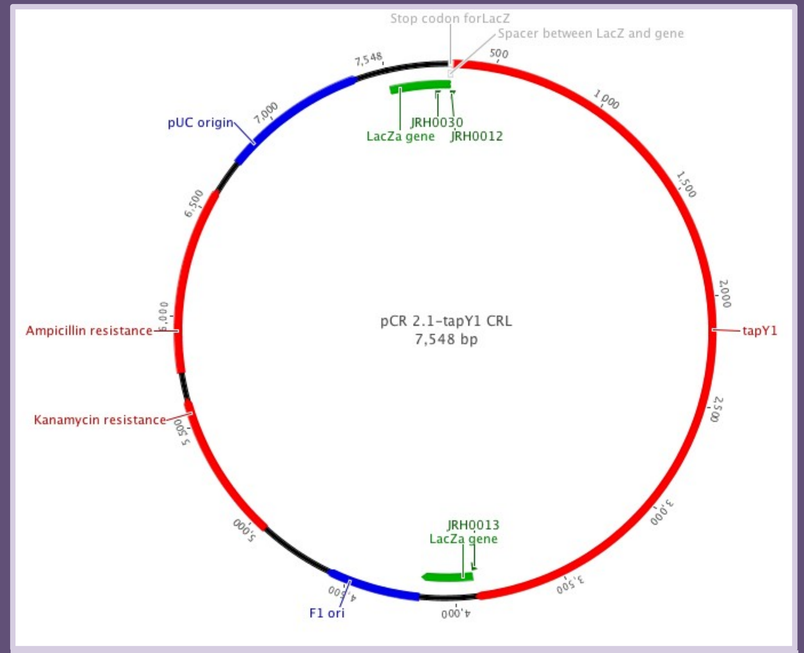

Figure 3. Map of pCR 2.1-*tapY1* CRL plasmid containing the introduced *tapY1* gene amplified from *A. salmonicida*

Materials and Methods, Continued.

Complementation Vector Construction. The *tapY1* gene sequence was amplified from the genome of *A. salmonicida* by means of PCR and was ligated into vector, pCR 2.1 (Life Technologies). A ligation reaction was carried out with a diluted PCR product containing the *tapY1* gene, the PCR 2.1 vector, 10X ligation buffer and T4 DNA ligase. This vector was then introduced into a strain of *E.coli* DH5α via calcium chloride transformation. PCR with the plasmid and JRH0012 and JRH0013 was carried out to confirm the presence of the *tapY1* gene. Another set of PCRs were set up with the plasmid and JRH0030 and JRH0013 as well as JRH0030 and JRH0012 to determine the orientation of the insert in the vector.

Results and Discussion

The construction of the SOE PCR fragment for creation of the *tapY1* mutant is still in progress. Product "AB" will be combined with product "C" to amplify the full "ABC" product. The completed "ABC" product from the splice overlap extension will be used to transform the wild type *A. salmonicida* into a mutant in which the *tapY1* gene will be replaced with the gentamycin resistance cassette (Figure 2). The mutant strain will then be tested for loss of the ability to perform natural transformation. Previous studies in the Huddleston lab with mutants with point mutations in *tapY1* show a loss of transformation, so it is assumed that a complete replacement will show the same results.

Assuming there is reduced or complete loss of natural transformation ability in the mutant, the next step is to confirm that the missing *tapY1* gene is responsible for the loss of transformation. A complementation vector (pCR 2.1-tapY1 CRL) (Figure 3) was successfully constructed. It will restore the gene sequence (with a lac promoter) to the mutant cells and allow for restored expression of *tapY1* in the mutant strain.

Conclusion

With a successful plasmid containing the *tapY1* gene, the rest of the project will focus solely on splicing together the "A","B",and "C" regions present in the wild type genome to create the desired mutant strain. With the mutant strain created, we can then introduce the gentamycin casette and asses the changes in transformation ability. After that has been observed, we will be able to fully reintroduce the *tapY1* gene back into the genome by introducing the mutant strain with the pCR 2.1 – *tapY1* plasmid. This will allow for continued insight into the horizontal gene transfer ability of *Aeromonas.*

Citations

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