Natural Transformation of the *tapY1* Gene Sequence in *Aeromonas salmonicida*

Kristin Goodwin and Jennifer Huddleston
Biology Department, Abilene Christian University, Abilene Texas 79699

Abstract

Natural transformation is an important research topic because it is the passing of genes horizontally between unrelated cells which allows microbial species to gain or lose certain characteristics, including antibiotic resistance. *Aeromonas* species exhibit natural transformation and the competency to incorporate foreign DNA into their genetic material. The Huddleston Lab is attempting to determine the gene products *Aeromonas* needs for this type of transfer. This report specifically describes the investigation of the *tapY1* gene product. The *tapY1* gene sequence encodes an extended type IV pili filament which allows for the ability of the cell to attach to adjacent cells and surfaces. It is hypothesized to play a role in natural transformation by binding to DNA. Primers flanking the *tapY1* gene sequence were created and used in order to amplify the gene by polymerase chain reaction (PCR). The amplified DNA was then sequenced and verified as the *tapY1* gene from *Aeromonas salmonicida*, confirming the preliminary strain identification. Knowing the specific DNA sequence of the region will allow for targeting of *tapY1* for replacement. This will result in the *Aeromonas salmonicida* strain losing the ability to for pili, while gaining the characteristics of the replacement genes: gentamycin resistance used for selection. This newly constructed strain in which the *tapY1* genes have been lost can then be screened for a change in its transformation ability.

Introduction

The genus *Aeromonas* represents gram-negative, rod-shaped bacteria that are commonly found in aquatic environments (1). Not only have certain species been noted to cause infections in fish and amphibians, but also human gastrointestinal, opportunistic wound, and nosocomial infections arise due to colonization of these species within the body (2, 3). Humans face the challenge of coming into contact with these potentially harmful species through outdoor recreational activities and ingesting infected consumable products (4,5). Individuals with compromised immune systems are more prone to contract the infections (6). So far, *Aeromonas* infections remain combatable by antibiotics (6). The question of whether *Aeromonas* species in the environment are capable of performing natural transformation arises, and can have a major effect on drug resistance in the future. Natural transformation is the uptake of free DNA horizontally from different species within the environment, not vertically from parent to offspring, and incorporating the new DNA within the bacterial genome (7). To determine whether this occurs, specific genes with notable functions must be analyzed within the *Aeromonas* genome. This research focuses on the *tapY1* gene sequence of *Aeromonas salmonicida*. Type IV pilin biogenesis protein, which the *tapY1* gene sequence encodes, plays a role in DNA binding and is noted for the presence of a pili ejecting from the cell. To study the effect of the *tapY1* gene on transformation, the gene sequence will be replaced with a gentamycin cassette. This will create a knock-out strain in which there is no longer *tapY1*. This mutant *Aeromonas salmonicida* strain will then be studied for a change in transformation capabilities.

Materials and Methods

The computer program, Geneious, allowed the designing of primers necessary to sequence the *tapY1* gene. Once, the primers were acquired, Polymerase Chain Reaction (PCR) was used to amplify the *tapY1* gene sequence, as well as specific regions determined by the use of different combinations of primers. The following cycling conditions were used: 1 cycle of 94°C for 1 min, 30 cycles of 94°C for 30 sec, 52.7°C for 30 sec, 72°C for 6 min , and 1 cycle of 72°C for 10 min. Amplification reactions contained 1 × Accutaq reaction buffer, 2.5m M of each dNTP, 0.5 µl of Accutaq DNA polymerase, 0.1 µM of each primer, and 40 ng of template DNA in a final reaction volume of 50µl. Primers for the gentamycin gene sequence were used to amplify this gene cassette. This step will allow for the *tapY1* gene sequence to be precisely removed and replaced by the entire gentamycin gene sequence. Splice Overlap Extension (SOE) PCR combines the gene sequences on either side of the *tapY1* gene sequence with the gentamycin gene sequence by using primers designed to form the transforming product with forward and reverse sequences from either side of the *tapY1* gene.

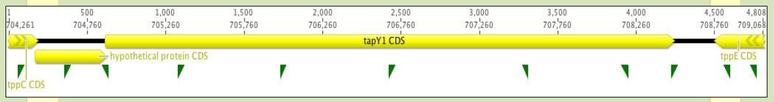

Figure 1: *tapY1* gene sequence constructed on Geneious and amplified by PCR.

Identification	Primer Sequence (5' - 3')	Temp (°C)	Purpose
KLG001	TCAACTGGGTAATGTCAGTGTG	54.8	The forward primer for amplifying the UP region of the tapY1
KLG002-1	CGCTGCCGCTCCTATTC	56.3	The reverse primer for amplifying the UP region of the tapY1
JRH0010	TTAGGTGGCGGTACTTGG	54.3	The forward primer for amplifying the gentamycin gene
KLG002	CCAAGTACCGCCACCTAACGCTGCCGCTCCTATTC	54.3/56.3	Anneals to the gentamycin gene/anneals to the UP region of the tapY1
JRH0011	ATGTTACGCAGCAGCAAC	53.7	The reverse primer for amplifying the gentamycin gene
KLG003-1	AAATGATCCTGTGGCTGATGG	55.3	The forward primer for amplifying the DOWN region of the tapY1
KLG003	GTTGCTGCTGCGTAACATAAATGATCCTGTGGCTGATGG	53.7/55.3	Anneals to the gentamycin gene/anneals to the DOWN region of the tapY1
KLG004	TGGTGTTCACTGACAGCAAC	55.6	The reverse primer for ampligying the DOWN region of the tapY1

Table 1: Splice Overlap Extension PCR primers for the "A," "B," and "C" regions

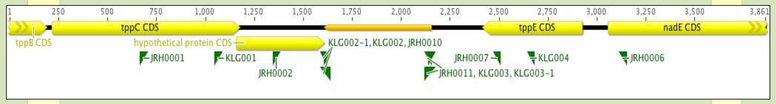

Figure 2: The complete SOE-PCR Product "ABC" spanning from KLG001 to KLG004. The orange section represents the gentamycin cassatte ("B").

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Results and Discussion

The entire *tapY1* gene sequence and flanking regions are contained between the forward JRH0001 and reverse JRH0006 primers (Fig. 1). This product is 5.5 kb long. The gene sequence was amplified using PCR with AccuTaq Polymerase. An NCBI Blast search, performed on March 19, 2013, revealed a 97% identity of the sequenced gene with the tapY1 gene sequence of *Aeromonas salmonicida*. The E-value calculated was 0.0, confirming the minimal differs between the sequence being studied and *A. salmonicida's* type IV pilus assembly protein gene region.

Primers were designed in the forward (JRH0010) and reverse (JRH0011) direction to amplify the gentamycin resistance cassette ("B") (Fig. 2, Table 1). The gentamycin cassette was successfully amplified, and currently SOE PCR is being used to construct a product that will knock-out the tapY1 gene in *Aeromonas salmonicida*. Primers for the flanking regions surrounding the *tapY1* gene sequence were constructed (Table 1). Both flanking regions were designed at 500 base pairs, ("A" and "C") while the gentamycin cassette ("B) is 534 base pairs. The first step of SOE PCR combined product "A" and "B" together using primers KLG001 (forward) and JRH0011 (reverse). The next step, which is in progress, combines the constructed product "AB" with product "C" to amplify the full "ABC" product (Fig. 2). The completed SOE-PCR construct "ABC" will be used to transform into the wild-type *A. salmonicida* in order to replace the *tapY1* gene with the gentamycin resistance cassette. The mutant will then be studied for loss of transformation ability.

Conclusion

For being a new research lab, the Huddleston Lab has made significant strides in microbiology research. *Aeromonas* is important pathogen due to their potential to laterally transfer genes associated with toxin production, biofilm formation, antibiotic resistance, and other virulence determinants. This is the beginning of understanding the significant process of natural transformation in *Aeromonas* since it previously has not been widely known or studied.

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