Cloning And Transformation Of *Pseudonomas aeruginosa* Azoreductase Gene

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Abstract

Pseudomonas aeroginosa (PA) is a gram negative aerobic rod that can cause serious, possibly life threatening, disease in humans, plants, and animals. In order to develop new and better treatments against PA, it is important to understand how this bacterium works to survive. Azoreductase from PA has quinone activity that aids the survival of PA, which can lead to an increase in antibiotic resistance. Using molecular biology approaches, we were able to successfully PCR amplify and clone the AzoR gene into a small, round strand of DNA called an expression plasmid vector (pET15b), transfer that plasmid into *E.coli* strain BL21, and then confirm that the E.coli were successfully transformed with the plasmid containing our gene of interest.

Keywords: *Pseudonomas aeruginosa*, Azoreductase gene, *E. coli*

Introduction

Pseudomonas aeruginosa can cause disease in humans, plants, and animals and is also a Gram negative aerobic rod bacterium. Aerobic bacteria survive in an oxygenated environment, thereby, it is found in most man made environments as well as, water, soil, and skin. *P. aeruginosa* infections usually occur into in people admitted into the hospital because they have weakened immune systems. *P. aeruginosa* is spread by direct human contact or touching equipment that is exposed and not properly cleaned. *P. aeruginosa* is a majorly adaptable human pathogen. During the last 20 years, *P. aeruginosa* has adapted by becoming resistant to the antibiotics and sterilizers that normally eliminate or kill the bacteria. According to Stover (2000), it is now a significant source of bacteria in burn victims, urinary-tract infections in patients with a catheter, and pneumonia in patients using respirators in hospitals. Usually, *P. aeruginosa* is treated with antibiotics; however, due to its antibiotic resistance, *P. aeruginosa* is considered an emerging "superbug". Therefore, there is a crucial need for new antibiotics. (Boucher *et al*. 2009). In order to develop new and better treatments against *P. aeruginosa*, it is important to understand the mechanism of how this bacterium survives. The DNA sequence is of interest to scientists, because it is an important bacterium as a pathogen, and it offers new information related to the size of the genome, genetic complexity and environmental adaptability, as well as its resistance. At 6.3 million base pairs, the *P. aeruginosa* genome is noticeably larger than most of the strains, totaling - 25 sequenced bacterial genomes (Ryan 2010). The enzyme azoreductase (AzoR), which is involved in the breaking down of azo dyes (dyes that contain nitrogen double bonded to nitrogen), may play a role in bacterial resistance to antibiotics. The *P. aeruginosa* stain PAO1 is known to possess up to three genes that code for AzoR activity (Ryan *et al*. 2010), as well as quinone oxidoreductase activity (Ryan *et al*. 2014). However, little data exist about whether this quinone reductase activity is involved in *P. aeruginosa*'s ability to survive and eventually develop antibiotic resistance. The current project is to identify new azoreductase genes from *P. aeruginosa* strain FRD1, which was isolated from a patient suffering from cystic fibrosis. The polymerase chain reaction (PCR) technique was used to amplify the PA2580 gene, a candidate azoreductase gene. The amplified gene was cloned into a pET15b expression vector and transformed into *Escherichia coli*. Cloning and transforming of the azoreductase gene will allow further studies related to the structure and function of the new azoreductase protein.

Methods

Bioinformatics Analysis

Using the National Library of Medicine's National Center for Biotechnology Information (NCBI) database, a putative azoreductase gene was identified from the *P. aeruginosa* genome (strain FRD1) that had high similar and identity homology to other azoreductases. The identified gene is

*=Mentor

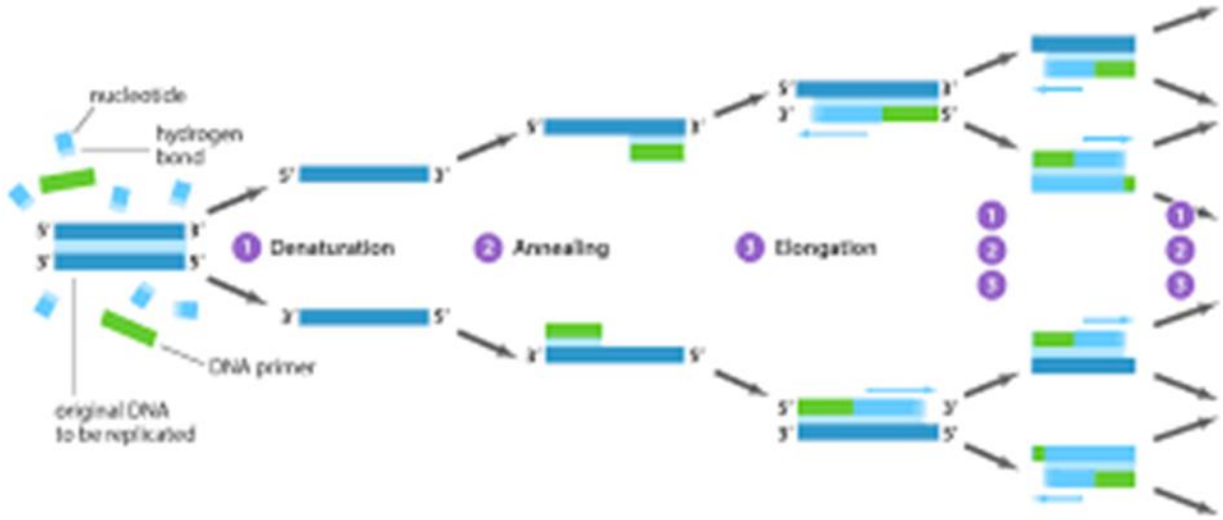

Figure 1 *- The PCR amplification process*

referred as PA2580, a putative AzoR in *P. aeruginosa*.

Chromosome extraction

A colony of *P. aeruginosa* was grown on a Trypticase soy agar (TSA) and used to inoculate a liquid broth (LB a nutrient medium). The overnight culture was used to carry out chromosomal DNA using a QIAamp DNA Mini Kit. An agarose gel was used to confirm purity of DNA, and the concentration of the DNA was determined using a spectrophotometer set at 260 nm.

Polymerase Chain Reaction (PCR)

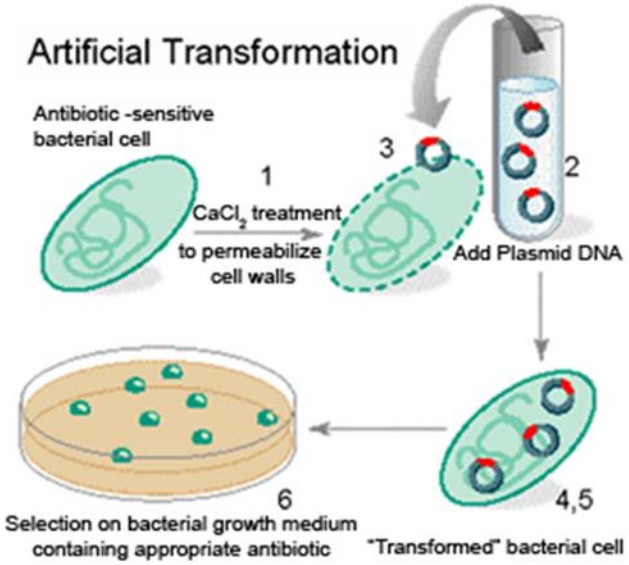

Figure 3 *- Steps used to clone the PCR amplified PA2508 gene and transform the recombinant plasmid into E. coli.*

This DNA sequence information was used to design short, single-stranded DNA "primers" for PCR amplification and cloning of the gene of interest (PA2580). The PCR technique was used to amplified a 591 base pair DNA via a thermocycler using heating and cooling cycles (Figure 1). During step one of the PCR cycles, the sample is heated to break the hydrogen bonds between the double-stranded DNA. Step two is cooled to allow primers to specifically bind to its complementary strand. Finally, the temperature is increased to allow a new DNA strand to be synthesized by the addition of bases (i.e. dNTPs). With each cycle the target sequence doubles.

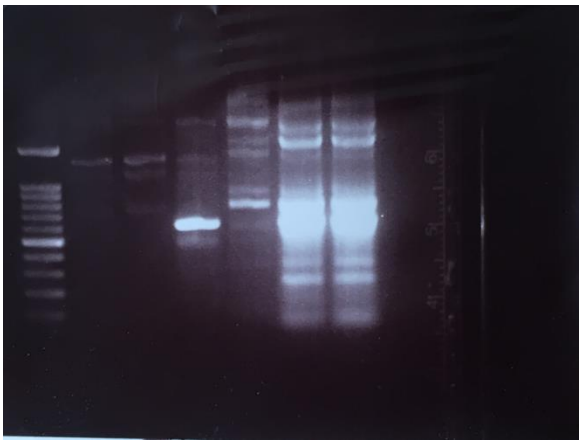

Figure 2 *- PCR Amplification of the PA2580 gene. PCR Amplification of the PA2580 ORF. Lane 1: Marker. Lane 2 and 3: negative samples Lane 4, 5, 6, and 7: PA2580 ORF. Arrow represents the 591 base pair PCR product.*

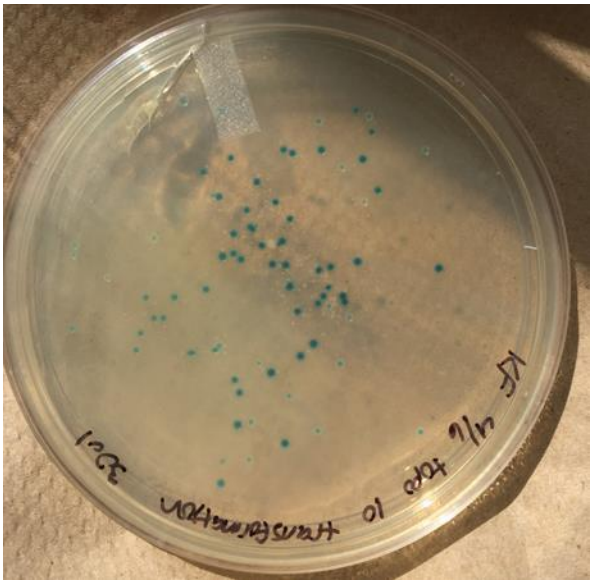

Figure 4 *- E. coli TOPO TA 10 transformants. The colonies with blue represent plasmid only, while the white colony represent plasmid containing the PA gene.*

Cloning and transformation

The amplified gene was purified using a gel electrophoresis kit (a method using positive electricity to separate negative material into components). Insertion (ligation) of the amplified gene into the plasmid is accomplished using "restriction enzymes" XhoI and BamHI, which were added to the forward and reverse primers prior to PCR amplification. The restriction enzymes simultaneously cut open the amplified PA2580 gene as well as the pET15b plasmid vector. The digested (cut) PCR was then ligated ("tied to" or "attached") into the cut pET15b vector. Transformation of the *E. coli* (BL21 strain) with the plasmid/insert was performed. The transformants, genetically altered *E. coli* now containing the *P. aeruginosa* gene were selected onto LB plates treated with Ampicillin. A colony PCR was then carried out to confirm clones with the correct insert.

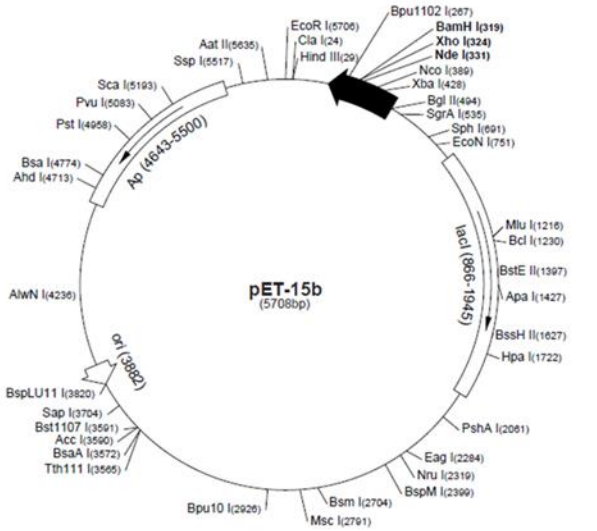

Figure 5 *- The pET-15b vector carries an N-terminal His Tag sequence at the N-terminal followed by a thrombin site and three cloning sites. XhoI and BamHI sites were used to clone the PA gene (see black arrow).*

Results

The PA2580 was successfully PCR amplified, cloned and transformed into *E. coli*, as represented in Figures 1, 2, 3 and 4. Figure 1 shows how the PA2580 gene was PCR amplified from the extracted chromosomal DNA. Figure 2 shows the process of transforming *E. coli* with the plasmid pET15b containing the PA2580 gene. *E. coli* will serve as the heterologous expression system to synthesize the PA2580 protein. Figure 3 shows the amplified PCR DNA (591 bp) using the primers generated (see blue arrow). Figure 4 shows the colonies of transformed *E. coli* Blue colonies represent plasmid only and white colonies represent plasmid and PA2580 gene insert (see blue arrow).

Discussion

The data show the successfully rescue, cloning, and transformation of a *P. aeruginosa* gene that may encode for azoreductase and quinone enzyme activity. This supports our hypothesis that additional azoreductase genes are likely present in the *P. aeruginosa* FRD1 strain. Future structure and function studies will be performed to express the pET15b/gene in *E. coli* BL21 cells and to purify the azoreductase protein using a technique known as Nickle chromatography. As shown in Figure 5, the expression vector (pET15b) will allow the expressed protein to be tagged with a series of histidines known as a His tags. Histidine tags on the protein allow the protein to be separated from other non-tag proteins. Once the protein is purified, enzymatic activity studies will be performed on the purified protein, specifically its ability to possess quinone reductase activity. In conclusion, the study makes it possible to eventually evaluate increased survivability and antibiotic resistance of *P. aeruginosa*.

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