**THE ROLE OF AlGB GENE ON THE ALGINATE PRODUCTION IN PSEUDOMONAS AERUGINOSA**

*P. aeruginosa* is an environmental gram-negative bacterium; however, it has the ability to cause lung infections and disease in patients with cystic fibrosis (CF). During this process, the bacterium converts into a slime material, called alginate, which forms a biofilm, known to be more resistant to antibiotics and host defenses. The genetic regulation of alginate is tightly controlled. The objective of this study was to investigate the role of an alginate-regulatory gene, *algB*.

This gene was first tested by the polymerase chain reaction (PCR) using clinical isolates of *P. aeruginosa* as template. We found that the majority of these bacteria carried the *algB* gene. To study whether the *algB* gene has a role in the slime production, we cloned this gene into a pCR-TOPO vector, which was then transferred into a *Pseudomonas* knockout vector. The inactivation of the *algB* gene was performed by transposon mutagenesis within this recombinant plasmid. The role of *algB* was tested by the inactivation of this gene within these isolates through homologous recombination via triparental conjugations. In conclusion, we found that *P. aeruginosa* has the *algB* gene, and this study determined how this gene affects the overproduction of alginate.

**INTRODUCTION**

Children with cystic fibrosis (CF) are particularly susceptible to fatal lung infections caused by an environmental bacterium called *P. aeruginosa*. During its chronic lung infections, this bacterium expresses unique growth characteristics such as over-production of slime resulting in a mucoid colony shape on a plate. Conversion to a mucoid shape is caused by overproduction of alginate by *P. aeruginosa*. This activity, coupled with the activation of the *algB* gene, encodes an activator required for the slime production. The genome of the standard reference strain of *P. aeruginosa*, PAO1, has recently been sequenced. However, the majority of the genes on it are still unknown (Figure 1).

**SPECIFIC AIMS**

This study was conducted for two purposes: 1) to achieve cloning and inactivating the *algB* gene on the *P. aeruginosa* genome; and 2) to determine whether the *algB* gene is one of the causes for the “slimy” colony on a plate.

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From the Joan C. Edwards School of Medicine and Marshall University; Huntington, West Virginia.
Fig 2. Effect of the inactivation of the algB gene on the mucoid colony shape in mucoid P. aeruginosa strains PAO1, PAO568, and PAO581. The picture shows the inactivation of the algB gene in PAO568 and PAO581 makes the bacterium change from a slimy into a non-slimy form.

METHODS

The following steps were taken to complete the study:
1. Polymerase chain reaction (PCR) allowed the amplification of the algB gene in many copies;
2. The algB gene was cloned into a pCR-TOPO vector from a commercial source;
3. The algB fragment was digested with a restriction enzyme called XbaI, producing a sticky XbaI tail on both ends of the algB gene;
4. This fragment was inserted into a P. aeruginosa suicide vector (pED6);
5. Blue/white screening was used to see if the right clone was obtained (white clone desired) in E. coli;
6. The recombinant plasmid (pED6-algB) was purified and treated with transposon mutagenesis to generate a library of tetracycline resistance colonies in E. coli;
7. This library was used as a donor source mated with the helper and the recipient cells, various non-mucoid and mucoid strains of P. aeruginosa;
8. The mated cells were incubated on a LB plate for 6 h at 37°C, which were then transferred onto a selective media called Pseudomonas isolation agar (PIA) supplemented with tetracycline. The growth of E. coli was inhibited but not the algB mutants of P. aeruginosa;
9. The expected mutants were further tested for the colony shape on a PIA plate by growing at 37°C overnight.

RESULTS

The algB gene was amplified by PCR and cloned into two vectors, pCR-TOPO and pED6. The algB gene was knockout on the genome by homologous recombination. The algB gene is required for slime production because inactivation of this gene on the genome makes mucoid P. aeruginosa converted into a non-mucoid form.

CONCLUSIONS

The algB gene contributes to the chronic lung infections in CF. The non-mucoid plates as shown in Figure 2 show that inactivation of the algB gene significantly reduces slime production. (Figure 2) Therefore, this might help CF patients fight against lung infections. In this study, all samples showed that the inactivation of the algB gene severely limits the slime production.

IMPLICATIONS

The algB gene should be considered as a new drug target to control the slime production in P. aeruginosa lung infections in CF. However, a limitation of this study is that the effect of the algB gene on the slime production in CF isolates of P. aeruginosa has not been tested.

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