# Role of the AcrAB-TolC Pump in Growth and Motility in *Escherichia coli*.

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## Abstract

Antibiotic resistant bacteria use multidrug efflux pumps such as the AcrAB-TolC of *Escherichia coli* in order to pump out antibiotics, metabolites, and other toxic compounds. AcrAB-TolC seems to also affect motility and other physiological functions. The purpose of our research is to understand the role of this pump and its regulators in growth and motility. Using a combination of growth and motility assays to test the effect of gene deletions of this pump, its regulators and flagellum genes, as well as of the pump substrate rhodamine and the pump inhibitor PAβN, we have found that PAβN decreases the growth of different pump and flagellum mutants. Our findings highlight the complex interplay between antibiotic resistance, cell growth and motility. These findings may contribute to long-term research to find more efficient ways to combat antibiotic resistance.

## Introduction

Antibiotic resistance is a developing issue in the fight against pathogenic bacteria. More and more bacteria are acquiring methods of resistance to antibiotics, such as by blocking penetration through the cell membrane, target modification, and efflux pumps. In our study we focus specifically on the AcrAB-TolC efflux pump. The AcrAB-TolC pump is a method for multidrug resistance used by *Escherichia coli.* It is able to be used for efflux of various types of compounds that can differ in shape, size, and chemical makeup. The components of the pump are the outer membrane channel, TolC, the secondary transporter, AcrB, and AcrA, which bridges the two other integral proteins (1). Two substrates focused on in this study were PAβN and Rhodamine. PAβN (Phenyl Arginine beta Naphthylamide) is an inhibitor of the pump that has also been found to permeabilize bacterial membranes. (2)When the AcrAB-TolC pump is affected, a change in motility of the bacteria can be observed, specifically when AcrB or AcrR are deleted. (3) For this reason, we are interested in determining the relationship between how the bacteria respond to different concentrations of substrates when various genes responsible for the pump are deleted, and growth changes with these conditions and flagellum gene deletions.

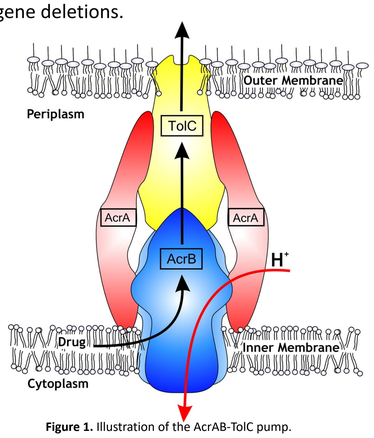
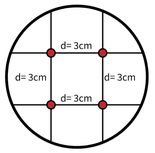



**Figure 1.** Illustration of the AcrAB-TolC pump.

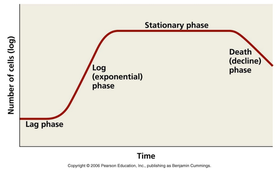
## Methodology

Motility Assay




- Plates are made of EZ Media which is a rich defined medium for bacterial growth.
- A consistent final agar concentration of 0.3% is used to facilitate swimming motility.
- Strains are poked underneath the surface, and are incubated at 37˚C.
- Diameters are measured once after 18 hours and once after 24 hours.

Growth Curve




- Ability to watch the detailed Optical Density (OD) and growth throughout 24 hours.
- Log Phase, also known as the exponential phase is important in our observations as it describes the speed at which different strains grows.
- A Microplate reader, the Spectra MAX 190 is used to read the 96 Well plates.

## Results

**Rhodamine Experiment Results:**


**Figure 2.** Gradient of increasing Rhodamine concentration in EZ Glucose Media plates with .3% Agar. Plates have been inoculated with four strains in the same organization on each plate: starting top left, clockwise: WT (wild type) is E. coli BW25113; ΔTolC pump knockout; ΔB: pump deletion; ΔR: pump regulator. Strains were obtained from Keio Collection.

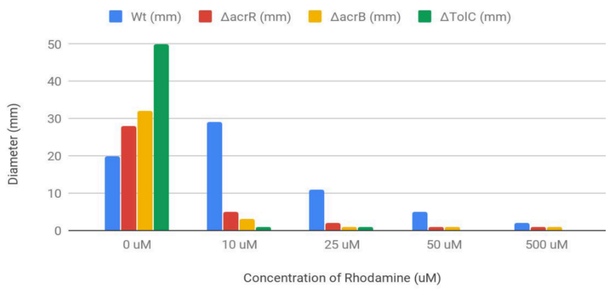



**Figure 3.** Diameter of Wild Type (WT), ΔacrR, ΔacrB and ΔTolC (in mm), vs concentration of Rhodamine (in µM).

**Diameter of Strains measured at various Rhodamine Concentrations**

| 24 hours | Wt (mm) | ΔacrR (mm) | ΔacrB (mm) | ΔTolC (mm) |
|---|---|---|---|---|
| **0 uM** | 20 | 28 | 32 | 50 |
| **10 uM** | 29 | 5 | 3 | 1 |
| **25 uM** | 11 | 2 | 1 | 1 |
| **50 uM** | 5 | 1 | 1 | 0 |
| **500 uM** | 2 | 1 | 1 | 0 |

**Table 1.** Table describing the Diameter of the four strains (listed in figure 2) with varying concentrations of Rhodamine after 24 of incubation at 37˚C, in EZ Glucose media, as shown in figure 3.

**PAβN Experiment Results:**

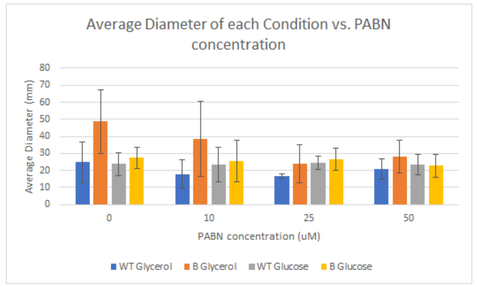



**Figure 4.** Measuring the diameter of BW 25113 Wild Type and ΔAcrB in EZ Glucose and EZ Glycerol at 37˚C, with an increasing concentration of PAβN. Each bar represents an average of 2-5 trials.

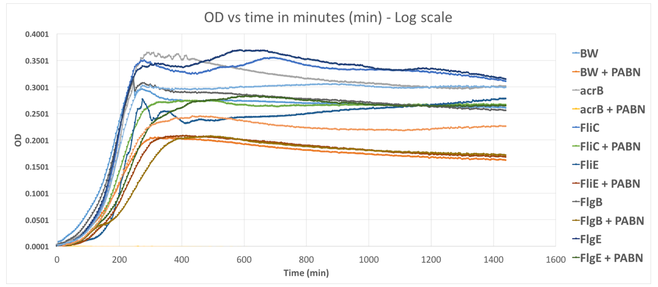



**Figure 5.** Growth curve depicting 6 Strains of E. coli; BW is the control E. coli strain, acrB is the strain with a deletion of the pump, FliC, FliE, FlgB, and FlgE are all genes related to function or structure of the flagellum. Growth rate was measured for each strain on its own, as well as with phenyl-arginine-beta-naphthylamide (PAβN) to a final Concentration of 250 µM.

## Conclusion

**Rhodamine Experiment Conclusions:**

We expect that when there is a deletion of the AcrAB-TolC pump, there could be an increase of motility due to lack of efflux of metabolites. W expect the strains tested to all decrease motility when substrates, such as Rhodamine, are added to the medium. Rhodamine is a substrate of the pump that could act as a competitive inhibitor; therefore, the higher the Rhodamine concentration, metabolites of the cell may be kept inside the cell for longer amount of time, which might be toxic to the cell at certain concentrations.

What we observed is an increase in the diameter of just the Wild Type strain when exposed to 10µM Rhodamine. This critical concentration seems to make the cell more motile. This result with the Wild Type could be indicative of a relationship between the efflux pumps and motility. The cell might be attempting the move away from highly concentrated areas using chemotaxis. As the motility assay can only look at motility, there is a need for measurement of growth using growth curves. We have results using PAβN, but to answer these questions, these experiments using Rhodamine need to be conducted.

The ΔTolC strain is most susceptible to the slightest concentration of Rhodamine. The TolC protein is the main protein of the pump which links the inner membrane of the cell to the outer membrane and eventually out of the cell. Removing ΔTolC will inactivate not only the AcrAB-TolC pump, but also a wide array of other pumps in the cell. This makes the cell unable to efflux out metabolites or Rhodamine, which is observed in its lack of growth.

**PAβN Experiment Conclusions:**

A similar effect was observed with PAβN as was observed with Rhodamine. PAβN ( phenyl-arginine-beta-naphthylamide) is a known inhibitor of the AcrAB-TolC pump, and was observed to drastically change the growth of E.coli. This was especially true in the ΔAcrB strain where adding a 250µM final PAβN concentration is enough to halt significant growth. The protein AcrB in another component of the pump which is in the inner membrane of the cell.

Our observations looked at both motility and growth by using flagellum mutants as well as pump mutants.

After getting growth curve results, we focused on ΔArcB at varying PAβN concentration in EZ Glucose and EZ Glycerol at 37˚C. We wanted to find the PAβN concentration threshold at which ΔAcrB stops growing, however, we have yet to find a trend, as shown in Figure 4.

## References

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