Exploring the Copper Homeostasis Networks in the Bacterium *Pseudomonas aeruginosa*

A Major Qualifying Project Submitted to the Faculty of WORCESTER POLYTECHNIC INSTITUTE In partial fulfillment of the requirements for the Degree in Biochemistry

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Report Submitted to:

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Abstract

Pseudomonas aeruginosa is member of the Gamma Proteobacteria class of bacteria, alongside other well-known bacteria like Salmonella, Escherichia coli, Shigella, Yersinia, and many more. It is a Gram-negative, aerobic rod belonging to the bacterial family Pseudomonadaceae, which includes the 12-member genus of *Pseudomonas*. What makes *P. aeruginosa* particularly interesting for studies is that it is a very antibiotic resistant and opportunistic bacteria, making it a dreaded and dangerous pathogen. Although the bacteria prefer to stay in soil and water, in the United States it has become the most common pathogen to cause nosocomial pneumonia and is frequently implicated in urinary tract and bloodstream infections. This is due to its presence in hospitals, but more specifically, the bacteria tend to create colonies on the medical equipment which then spreads to all kinds of patients. Another intriguing aspect of *P. aeruginosa* is its ability to uptake and efflux copper. To better understand the bacteria's structure, copper must be introduced in different concentrations to test whether the bacteria is tolerant to the metal. Different mutations can show different results to the introduction of copper, but with this method, each protein can be mapped and described for its function in the copper transportation system for the bacteria.

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Introduction

Pseudomonas aeruginosa is an asporogenous, monoflagellated and rod-shaped bacterium. It is classified as a Gram-negative opportunistic pathogen because of its ability to cause disease in plants, animals, and humans through infection. It is known to grow in a plethora of conditions, such as soil, water, and soap. It grows well within the range of 25°C to 37°C but is known to grow at 42°C; distinguishing it from many other *Pseudomonas* species. Its growth on surfaces is thanks to its ability to form biofilms: complex structures correlated with an increase in antibiotic resistance. *P. aeruginosa* is present in hospitals as well, being responsible for more than 10% of patient infections, especially in cystic fibrosis cases where the bacteria forms biofilm in the lungs. It is also considered to be a model organism for understanding other Gram-negative bacteria. Consequently, the study of *P. aeruginosa* is crucial in the fight against nosocomial infections; of which 50% are due to bacterial exposure.

Copper, a transition metal and the 29th element, is utilized in bacteria for the catalysis of electron transfer reactions. Although it serves its purpose in these reactions, copper can build up and develop oxidative stress within iron-sulfur clusters. Copper concentrations, however, are balanced to fulfill the catalytic needs of the bacteria, without causing any toxic effects. This balance is accomplished through a network of proteins that act as transport entries and exits, as well as chaperones, moving copper ions throughout the periplasm and cytoplasm. Mutations of proteins in the bacteria's network allows for a greater understanding of how the network functions. Without certain proteins, the network ceases to function properly and the bacteria dies due to copper toxicity.

Although many *P. aeruginosa* mutants are known, their function within the network is a mystery. Through this project the ability of mutants to survive in the presence of different copper concentrations is explored to find the phenotypes that represent valuable proteins in the network. If the mutated bacteria manage to survive in the presence of toxic copper concentrations, then the protein that was mutated was not particularly important since the wild type (PA01) of *P. aeruginosa* can survive, grow, and multiply under concentrations under 5 mM. The most well-known mutant is PA 3920; ΔCopA. This mutant cannot survive under any copper stress, creating a new control of what a crucial mutant is within the genomic sequence. Using growth curves of the mutants under copper concentrations marked at 0, 0.5, 1, 2, 3, and 5 mM, a wide range of mutants are tested and compared to their wild type and PA 3920 controls. The list of mutants used to explore these ideas is under appendix A.

Materials & Methods

Preculture of Bacterium

Pseudomonas aeruginosa cells taken from their TSA (trypticase soy agar) solid media plaque were inoculated into TSA liquid media with their corresponding antibiotic; PA01 wild type was treated with $25\,\frac{\mu g}{mL}$ of Irgasan, while PA mutants were treated with $5\,\frac{\mu g}{mL}$ of Tetracycline. These precultures were then grown overnight in a 37°C incubator; best if grown for roughly 18 hours to avoid any biofilm build up which can affect OD_{600} readings. Placed 1 mL samples from each preculture in absorbance tubes and to check for each preculture's absorbance, which will be later used for the 96-well plate preparations. Precultures were the quickest steps to the experiment, but due to the use of antibiotics, serious care was taken to ensure that it was correctly set up every time. Any samples thought to be contaminated or set up with incorrect measurements were immediately and appropriately disposed of.

96-Well Plate Set-Up

For this experiment, 96-well plates were used to maximize the amount of *P. aeruginosa* mutants and copper concentrations tested at a time. Each well was filled with 200 µL of a combination of 100 µL of TSA liquid media with *P. aeruginosa* (from the 1 mL samples of preculture) and 100 µL of TSA liquid media with varying copper concentrations. To mix the precultured *P. aeruginosa* samples with the liquid media and copper mixtures, 15 mL tubes were filled with roughly 3 mL of new liquid TSA media, 75 µg of Irgasan, and the necessary volume of copper (II) sulfate to reach the desired concentration. To ensure that each well contained the proper concentration of copper, each tube was provided with double the concentration of the desired final concentration; since placed into the wells, the concentration is halved due to the remaining of the well having to be filled with the precultured mutants and wild type. The CuSO4 additions according to concentration in the 15 mL tubes went as follows:

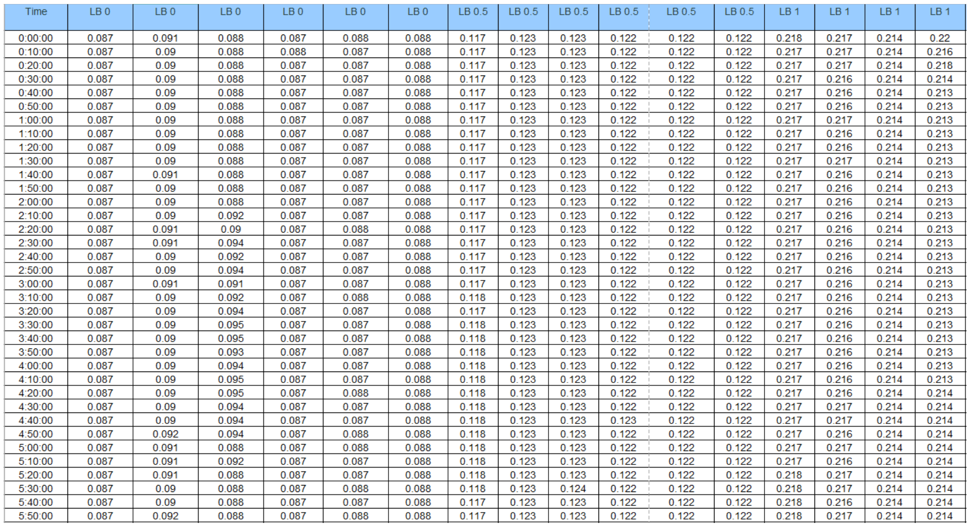
- 0.5 mM tube required 15 µL of 200 M $CuSO_4$
- 1 mM tube required 30 µL of 200 M $CuSO_4$
- 2 mM tube required 60 µL of 200 M $CuSO_4$
- 3 mM tube required 90 µL of 200 M $CuSO_4$
- 5 mM tube required 150 µL of 200 M $CuSO_4$

The mapping of the well plates was designed to contain an outer layer of bacteria-less wells that still included the liquid media with Irgasan and the different copper concentrations to maintain even heat distribution across the well plate. Each column within the mentioned control border was grouped with the same mutant of *P. aeruginosa*, usually paired in 2’s or 3’s directly adjacent to each other. Furthermore, each row contained the same concentration of copper while increasing from 0 to 5 mM from row B to G, respectively.

	1	2	3	4	5	6	7	8	9	10	11	12
A	LB 0	LB 0	LB 0	LB 0	LB 0	LB 0	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 0.5
B	LB 1	PA 01 0 mM	PA 01 0 mM	PA 2397 0 mM	PA 2397 0 mM	PA 3516 0 mM	PA 3516 0 mM	PA 2520 0 mM	PA 2520 0 mM	PA 3790 0 mM	PA 3790 0 mM	LB 2
C	LB 1	PA 01 0.5 mM	PA 01 0.5 mM	PA 2397 0.5 mM	PA 2397 0.5 mM	PA 3516 0.5 mM	PA 3516 0.5 mM	PA 2520 0.5 mM	PA 2520 0.5 mM	PA 3790 0.5 mM	PA 3790 0.5 mM	LB 2
D	LB 1	PA 01 1 mM	PA 01 1 mM	PA 2397 1 mM	PA 2397 1 mM	PA 3516 1 mM	PA 3516 1 mM	PA 2520 1 mM	PA 2520 1 mM	PA 3790 1 mM	PA 3790 1 mM	LB 2
E	LB 1	PA 01 2 mM	PA 01 2 mM	PA 2397 2 mM	PA 2397 2 mM	PA 3516 2 mM	PA 3516 2 mM	PA 2520 2 mM	PA 2520 2 mM	PA 3790 2 mM	PA 3790 2 mM	LB 2
F	LB 1	PA 01 3 mM	PA 01 3 mM	PA 2397 3 mM	PA 2397 3 mM	PA 3516 3 mM	PA 3516 3 mM	PA 2520 3 mM	PA 2520 3 mM	PA 3790 3 mM	PA 3790 3 mM	LB 2
G	LB 1	PA 01 5 mM	PA 01 5 mM	PA 2397 5 mM	PA 2397 5 mM	PA 3516 5 mM	PA 3516 5 mM	PA 2520 5 mM	PA 2520 5 mM	PA 3790 5 mM	PA 3790 5 mM	LB 2
H	LB 3	LB 3	LB 3	LB 3	LB 3	LB 3	LB 5	LB 5	LB 5	LB 5	LB 5	LB 5

Table #1: Example of a well map used in the experiment. The outer layer contained no bacteria while the inside consisted of different combinations of bacteria and copper.

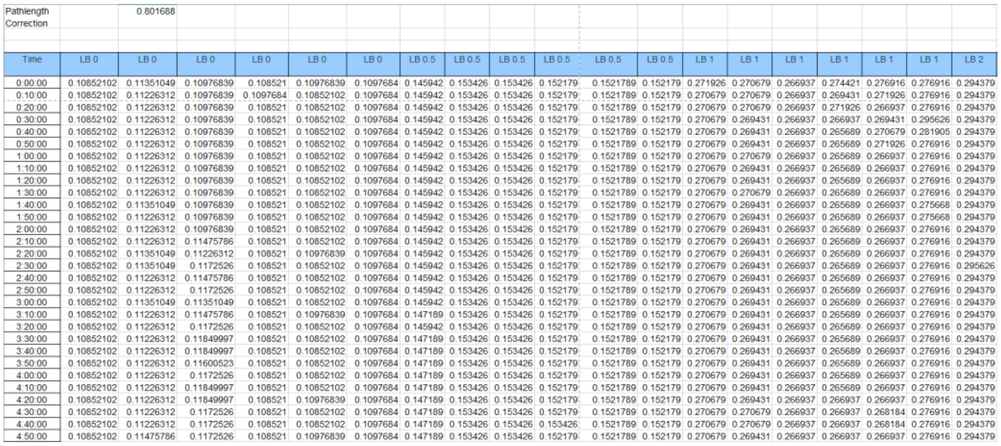
Overnight Growth Curve Corrections and Graphs. Once the well plate was set up, using the OD600 Program, the plate went into the OD600 MACHINE for 18 hours at 37°C. The program was set up take readings every 10 minutes to ensure that there were enough data points taken to create a continuous graph of each well. On the program the outer layers (rows A and H and columns 1 and 12) were labeled as controls, while the wells inside were labeled as experiments. Once the plate underwent its overnight growth, the program gave raw data that was moved into an excel file where it would be corrected for accuracy.



Time	LB 0	LB 0	LB 0	LB 0	LB 0	LB 0	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 1	LB 1	LB 1	LB 1
0:00:00	0.087	0.091	0.088	0.087	0.088	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.218	0.217	0.214	0.22
0:10:00	0.087	0.09	0.088	0.088	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.217	0.214	0.216
0:20:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.217	0.214	0.218
0:30:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.214
0:40:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
0:50:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
1:00:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.217	0.214	0.213
1:10:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
1:20:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
1:30:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.217	0.214	0.213
1:40:00	0.087	0.091	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
1:50:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
2:00:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
2:10:00	0.087	0.09	0.092	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
2:20:00	0.087	0.091	0.09	0.087	0.088	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
2:30:00	0.087	0.091	0.094	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
2:40:00	0.087	0.09	0.092	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
2:50:00	0.087	0.09	0.094	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
3:00:00	0.087	0.091	0.091	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
3:10:00	0.087	0.09	0.092	0.087	0.088	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
3:20:00	0.087	0.09	0.094	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
3:30:00	0.087	0.09	0.095	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
3:40:00	0.087	0.09	0.095	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
3:50:00	0.087	0.09	0.093	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
4:00:00	0.087	0.09	0.094	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
4:10:00	0.087	0.09	0.095	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.213
4:20:00	0.087	0.09	0.095	0.087	0.088	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.214
4:30:00	0.087	0.09	0.094	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.217	0.214	0.214
4:40:00	0.087	0.09	0.094	0.087	0.087	0.088	0.118	0.123	0.123	0.123	0.122	0.122	0.217	0.217	0.214	0.214
4:50:00	0.087	0.092	0.094	0.087	0.088	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.214
5:00:00	0.087	0.091	0.088	0.087	0.088	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.217	0.214	0.214
5:10:00	0.087	0.091	0.092	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.217	0.216	0.214	0.214
5:20:00	0.087	0.091	0.088	0.087	0.087	0.088	0.118	0.123	0.123	0.122	0.122	0.122	0.218	0.217	0.214	0.214
5:30:00	0.087	0.09	0.088	0.087	0.088	0.088	0.118	0.123	0.124	0.122	0.122	0.122	0.218	0.217	0.214	0.214
5:40:00	0.087	0.09	0.088	0.087	0.087	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.218	0.216	0.214	0.214
5:50:00	0.087	0.092	0.088	0.087	0.088	0.088	0.117	0.123	0.123	0.122	0.122	0.122	0.218	0.217	0.214	0.214

Table #2: Example of raw data exported into Excel with time parameter set to end at 18:00:00, and data included the rest of the wells (not shown).

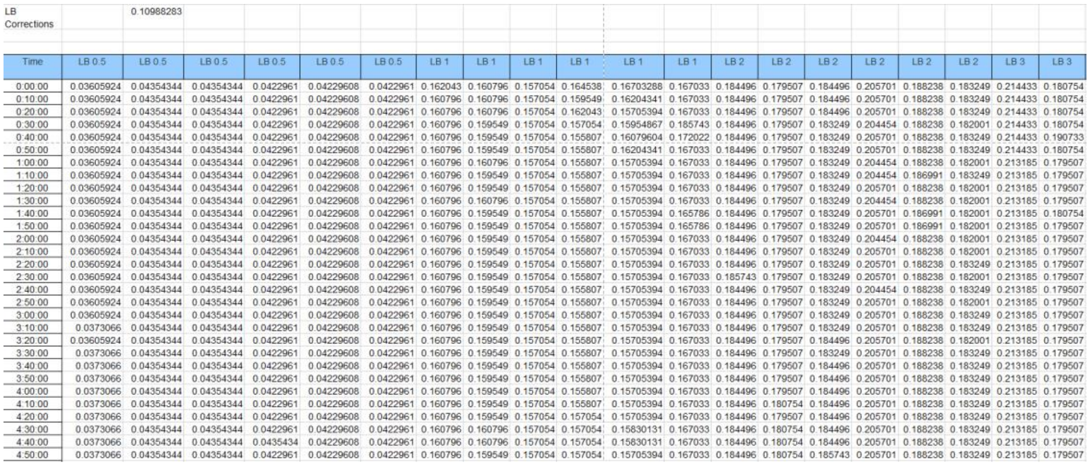
Corrections were necessary for the Excel sheets to ensure that the data was as accurate and precise as possible. The first correction was for the pathlength between the machine's light reader and the start of the wells. The program gave "read pathlength" data so the average was taken from all read pathlengths given. Then the raw data given for all 96 well plates was divided by the average of the read pathlengths. The correction ensured that the data was interpreted correctly for all the wells since the values for the pathlength varied just enough to create different readings per well.



Pathlength Correction		0.801688																	
Time	**LB 0**	**LB 0**	**LB 0**	**LB 0**	**LB 0**	**LB 0**	**LB 0.5**	**LB 0.5**	**LB 0.5**	**LB 0.5**	**LB 0.5**	**LB 0.5**	**LB 1**	**LB 1**	**LB 1**	**LB 1**	**LB 1**	**LB 1**	**LB 2**
0:00:00	0.10852102	0.11351049	0.10976839	0.108521	0.10976839	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.271926	0.270679	0.266937	0.274421	0.276916	0.276916	0.294379
0:10:00	0.10852102	0.11226312	0.10976839	0.1097684	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.270679	0.266937	0.269431	0.271926	0.276916	0.294379
0:20:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.270679	0.266937	0.271926	0.266937	0.276916	0.294379
0:30:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.266937	0.269431	0.295626	0.294379
0:40:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.270679	0.281905	0.294379
0:50:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.271926	0.276916	0.294379
1:00:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.270679	0.266937	0.265689	0.266937	0.276916	0.294379
1:10:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
1:20:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
1:30:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.270679	0.266937	0.265689	0.266937	0.276916	0.294379
1:40:00	0.10852102	0.11351049	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.275668	0.294379
1:50:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.275668	0.294379
2:00:00	0.10852102	0.11226312	0.10976839	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
2:10:00	0.10852102	0.11226312	0.11475786	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
2:20:00	0.10852102	0.11351049	0.11226312	0.108521	0.10976839	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
2:30:00	0.10852102	0.11351049	0.1172526	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.295626
2:40:00	0.10852102	0.11226312	0.11475786	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
2:50:00	0.10852102	0.11226312	0.1172526	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
3:00:00	0.10852102	0.11351049	0.11351049	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
3:10:00	0.10852102	0.11226312	0.11475786	0.108521	0.10976839	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
3:20:00	0.10852102	0.11226312	0.1172526	0.108521	0.10852102	0.1097684	0.145942	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
3:30:00	0.10852102	0.11226312	0.11849997	0.108521	0.10852102	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
3:40:00	0.10852102	0.11226312	0.11849997	0.108521	0.10852102	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
3:50:00	0.10852102	0.11226312	0.11600523	0.108521	0.10852102	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
4:00:00	0.10852102	0.11226312	0.1172526	0.108521	0.10852102	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
4:10:00	0.10852102	0.11226312	0.11849997	0.108521	0.10852102	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.265689	0.266937	0.276916	0.294379
4:20:00	0.10852102	0.11226312	0.11849997	0.108521	0.10976839	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.266937	0.266937	0.276916	0.294379
4:30:00	0.10852102	0.11226312	0.1172526	0.108521	0.10852102	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.270679	0.266937	0.266937	0.268184	0.276916	0.294379
4:40:00	0.10852102	0.11226312	0.1172526	0.108521	0.10852102	0.1097684	0.147189	0.153426	0.153426	0.153426	0.1521789	0.152179	0.270679	0.270679	0.266937	0.266937	0.268184	0.276916	0.294379
4:50:00	0.10852102	0.11475786	0.1172526	0.108521	0.10976839	0.1097684	0.147189	0.153426	0.153426	0.152179	0.1521789	0.152179	0.270679	0.269431	0.266937	0.266937	0.266937	0.276916	0.294379

Table #3: Example of an average pathlength represented by the cell containing 0.801688, the data shown was the result of the raw data divided by the pathlength average.

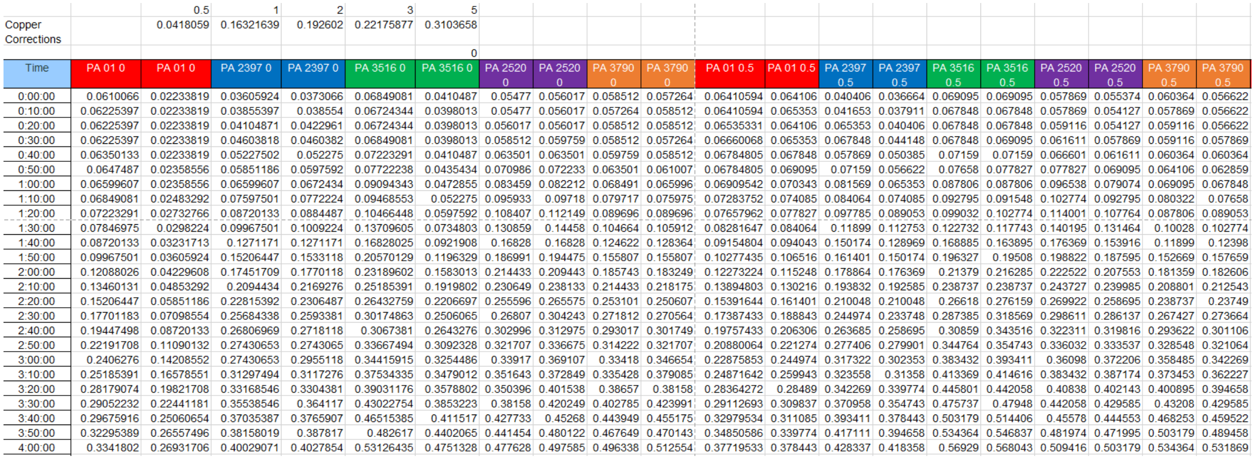
The next correction started by averaging all the data points for the wells that contained no bacterium or copper; they were labeled as LB 0 for liquid broth with 0 mM copper. This average can now be used as the liquid broth correction which was subtracted from all the remaining data points. This was done to make sure that the absorption reading given by the OD600 machine was more precise to just the bacterium and the copper, rather than the bacterium, copper, and the media affecting the absorption.



LB Corrections		0.10988283																		
Time	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 0.5	LB 1	LB 1	LB 1	LB 1	LB 1	LB 1	LB 2	LB 2	LB 2	LB 2	LB 2	LB 2	LB 3	LB 3
0:00:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.162043	0.160796	0.157054	0.164538	0.16703288	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.183249	0.214433	0.180754
0:10:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.160796	0.157054	0.159549	0.16204341	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.183249	0.214433	0.180754
0:20:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.160796	0.157054	0.162043	0.15705394	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.183249	0.214433	0.180754
0:30:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.157054	0.15954867	0.185743	0.184496	0.179507	0.183249	0.204454	0.188238	0.182001	0.214433	0.180754
0:40:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.16079604	0.172022	0.184496	0.179507	0.183249	0.205701	0.188238	0.183249	0.214433	0.190733
0:50:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.16204341	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.183249	0.214433	0.180754
1:00:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.160796	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.204454	0.188238	0.182001	0.213185	0.179507
1:10:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.204454	0.186991	0.183249	0.213185	0.179507
1:20:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.182001	0.213185	0.179507
1:30:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.160796	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.204454	0.188238	0.182001	0.213185	0.179507
1:40:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.165786	0.184496	0.179507	0.183249	0.205701	0.186991	0.182001	0.213185	0.180754
1:50:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.165786	0.184496	0.179507	0.183249	0.205701	0.186991	0.182001	0.213185	0.179507
2:00:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.204454	0.188238	0.182001	0.213185	0.179507
2:10:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.182001	0.213185	0.179507
2:20:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.183249	0.213185	0.179507
2:30:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.185743	0.179507	0.183249	0.205701	0.188238	0.182001	0.213185	0.179507
2:40:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.204454	0.188238	0.183249	0.213185	0.179507
2:50:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.182001	0.213185	0.179507
3:00:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.183249	0.213185	0.179507
3:10:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.183249	0.213185	0.179507
3:20:00	0.03605924	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.182001	0.213185	0.179507
3:30:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.183249	0.205701	0.188238	0.183249	0.213185	0.179507
3:40:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.183249	0.213185	0.179507
3:50:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.183249	0.213185	0.179507
4:00:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.183249	0.213185	0.179507
4:10:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.155807	0.15705394	0.167033	0.184496	0.180754	0.184496	0.205701	0.188238	0.183249	0.213185	0.179507
4:20:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.157054	0.15705394	0.167033	0.184496	0.179507	0.184496	0.205701	0.188238	0.183249	0.213185	0.179507
4:30:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.160796	0.157054	0.157054	0.15830131	0.167033	0.184496	0.180754	0.184496	0.205701	0.188238	0.183249	0.213185	0.179507
4:40:00	0.0373066	0.04354344	0.04354344	0.0435434	0.04229608	0.0422961	0.160796	0.160796	0.157054	0.157054	0.15830131	0.167033	0.184496	0.180754	0.184496	0.205701	0.188238	0.183249	0.213185	0.179507
4:50:00	0.0373066	0.04354344	0.04354344	0.0422961	0.04229608	0.0422961	0.160796	0.159549	0.157054	0.157054	0.15705394	0.167033	0.184496	0.180754	0.185743	0.205701	0.188238	0.183249	0.213185	0.179507

Table #4: Example of the LB corrections done; the average of the LB 0 data represented by the 0.10988283. Data shown resulted from the subtraction by LB correction.

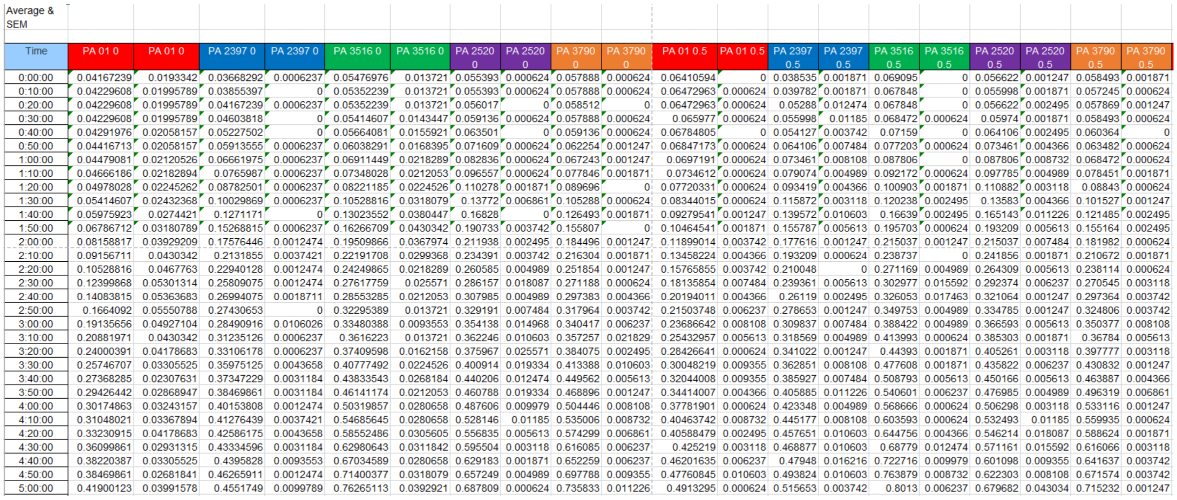
At this point the charts only included LB 0.5 through LB 5 with the mutants and wild type, so the next correction was set up by averaging the data points individually for each copper concentration. Once the 0.5 mM, 1 mM, 2 mM, 3 mM, and 5 mM were averaged, they were subtracted from their corresponding mutant data (i.e., PA 01 0.5 mM – 0.5 mM average). This step was crucial since it would finally give the absorption reading for the mutants and the wild type alone without any extra noise created by the media and copper.



		0.5	1	2	3	5														
Copper Corrections		0.0418059	0.16321639	0.192602	0.22175877	0.3103658														
						0														
Time	PA 01 0	PA 01 0	PA 2397 0	PA 2397 0	PA 3516 0	PA 3516 0	PA 2520 0	PA 2520 0	PA 3790 0	PA 3790 0	PA 01 0.5	PA 01 0.5	PA 2397 0.5	PA 2397 0.5	PA 3516 0.5	PA 3516 0.5	PA 2520 0.5	PA 2520 0.5	PA 3790 0.5	PA 3790 0.5
0:00:00	0.0610066	0.02233819	0.03605924	0.0373066	0.06849081	0.0410487	0.05477	0.056017	0.058512	0.057264	0.06410594	0.064106	0.040406	0.036664	0.069095	0.069095	0.057869	0.055374	0.060364	0.056622
0:10:00	0.06225397	0.02233819	0.03855397	0.038554	0.06724344	0.0398013	0.05477	0.056017	0.057264	0.058512	0.06410594	0.065353	0.041653	0.037911	0.067848	0.067848	0.057869	0.054127	0.057869	0.056622
0:20:00	0.06225397	0.02233819	0.04104871	0.0422961	0.06724344	0.0398013	0.056017	0.056017	0.058512	0.058512	0.06535331	0.064106	0.065353	0.040406	0.067848	0.067848	0.059116	0.054127	0.059116	0.056622
0:30:00	0.06225397	0.02233819	0.04603818	0.0460382	0.06849081	0.0398013	0.058512	0.059759	0.058512	0.057264	0.06660068	0.065353	0.067848	0.044148	0.067848	0.069095	0.061611	0.057869	0.059116	0.057869
0:40:00	0.06350133	0.02233819	0.05227502	0.052275	0.07223291	0.0410487	0.063501	0.063501	0.059759	0.058512	0.06784805	0.067848	0.057869	0.050385	0.07159	0.07159	0.066601	0.061611	0.060364	0.060364
0:50:00	0.0647487	0.02358556	0.05851186	0.0597592	0.07722238	0.0435434	0.070986	0.072233	0.063501	0.061007	0.06784805	0.069095	0.07159	0.056622	0.07658	0.077827	0.077827	0.069095	0.064106	0.062859
1:00:00	0.06599607	0.02358556	0.06599607	0.0672434	0.09094343	0.0472855	0.083459	0.082212	0.068491	0.065996	0.06909542	0.070343	0.081569	0.065353	0.087806	0.087806	0.096538	0.079074	0.069095	0.067848
1:10:00	0.06849081	0.02483292	0.07597501	0.0772224	0.09468553	0.052275	0.095933	0.09718	0.079717	0.075975	0.07283752	0.074085	0.084064	0.074085	0.092795	0.091548	0.102774	0.092795	0.080322	0.07658
1:20:00	0.07223291	0.02732766	0.08720133	0.0884487	0.10466448	0.0597592	0.108407	0.112149	0.089696	0.089696	0.07657962	0.077827	0.097785	0.089053	0.099032	0.102774	0.114001	0.107764	0.087806	0.089053
1:30:00	0.07846975	0.0298224	0.09967501	0.1009224	0.13709605	0.0734803	0.130859	0.14458	0.104664	0.105912	0.08281647	0.084064	0.11899	0.112753	0.122732	0.117743	0.140195	0.131464	0.10028	0.102774
1:40:00	0.08720133	0.03231713	0.1271171	0.1271171	0.16828025	0.0921908	0.16828	0.16828	0.124622	0.128364	0.09154804	0.094043	0.150174	0.128969	0.168885	0.163895	0.176369	0.153916	0.11899	0.12398
1:50:00	0.09967501	0.03605924	0.15206447	0.1533118	0.20570129	0.1196329	0.186991	0.194475	0.155807	0.155807	0.10277435	0.106516	0.161401	0.150174	0.196327	0.19508	0.198822	0.187595	0.152669	0.157659
2:00:00	0.12088026	0.04229608	0.17451709	0.1770118	0.23189602	0.1583013	0.214433	0.209443	0.185743	0.183249	0.12273224	0.115248	0.178864	0.176369	0.21379	0.216285	0.222522	0.207553	0.181359	0.182606
2:10:00	0.13460131	0.04853292	0.2094434	0.2169276	0.25185391	0.1919802	0.230649	0.238133	0.214433	0.218175	0.13894803	0.130216	0.193832	0.192585	0.238737	0.238737	0.243727	0.239985	0.208801	0.212543
2:20:00	0.15206447	0.05851186	0.22815392	0.2306487	0.26432759	0.2206697	0.255596	0.265575	0.253101	0.250607	0.15391644	0.161401	0.210048	0.210048	0.26618	0.276159	0.269922	0.258695	0.238737	0.23749
2:30:00	0.17701183	0.07098554	0.25684338	0.2593381	0.30174863	0.2506065	0.26807	0.304243	0.271812	0.270564	0.17387433	0.188843	0.244974	0.233748	0.287385	0.318569	0.298611	0.286137	0.267427	0.273664
2:40:00	0.19447498	0.08720133	0.26806969	0.2718118	0.3067381	0.2643276	0.302996	0.312975	0.293017	0.301749	0.19757433	0.206306	0.263685	0.258695	0.30859	0.343516	0.322311	0.319816	0.293622	0.301106
2:50:00	0.22191708	0.11090132	0.27430653	0.2743065	0.33667494	0.3092328	0.321707	0.336675	0.314222	0.321707	0.20880064	0.221274	0.277406	0.279901	0.344764	0.354743	0.336032	0.333537	0.328548	0.321064
3:00:00	0.2406276	0.14208552	0.27430653	0.2955118	0.34415915	0.3254486	0.33917	0.369107	0.33418	0.346654	0.22875853	0.244974	0.317322	0.302353	0.383432	0.393411	0.36098	0.372206	0.358485	0.342269
3:10:00	0.25185391	0.16578551	0.31297494	0.3117276	0.37534335	0.3479012	0.351643	0.372849	0.335428	0.379085	0.24871642	0.259943	0.323558	0.31358	0.413369	0.414616	0.383432	0.387174	0.373453	0.362227
3:20:00	0.28179074	0.19821708	0.33168546	0.3304381	0.39031176	0.3578802	0.350396	0.401538	0.38657	0.38158	0.28364272	0.28489	0.342269	0.339774	0.445801	0.442058	0.40838	0.402143	0.400895	0.394658
3:30:00	0.29052232	0.22441181	0.35538546	0.364117	0.43022754	0.3853223	0.38158	0.420249	0.402785	0.423991	0.29112693	0.309837	0.370958	0.354743	0.475737	0.47948	0.442058	0.429585	0.43208	0.429585
3:40:00	0.29675916	0.25060654	0.37035387	0.3765907	0.46515385	0.411517	0.427733	0.45268	0.443949	0.455175	0.32979534	0.311085	0.393411	0.378443	0.503179	0.514406	0.45578	0.444553	0.468253	0.459522
3:50:00	0.32295389	0.26557496	0.38158019	0.387817	0.482617	0.4402065	0.441454	0.480122	0.467649	0.470143	0.34850586	0.339774	0.417111	0.394658	0.534364	0.546837	0.481974	0.471995	0.503179	0.489458
4:00:00	0.3341802	0.26931706	0.40029071	0.4027854	0.53126435	0.4751328	0.477628	0.497585	0.496338	0.512554	0.37719533	0.378443	0.428337	0.418358	0.56929	0.568043	0.509416	0.503179	0.534364	0.531869

Table #5: Example of copper corrections shown under their corresponding labels.

By now only the data from wells B2 to G11 should remain in the spreadsheet. The final work done in Excel for the data was to get the averages and SEM’s (standard error of mean) of each bacterial well. This was done in the 10-minute intervals given from the OD$_{600}$ readings. Each pair, or set of 3, was averaged and placed into its own column (i.e., the 0-minute readings for the 2 PA 01 0 mM were averaged). Using the same grouping selection, the SEM was also found using the equation $\frac{\text{Standard Deviation of } (\#)}{\text{Square Root of } (\text{The \# of units included })}$. This data set was then considered the final data. As exemplified below, the final data was transferred into the GraphPad Prism 7 application where it was converted into individual graphs, organized by their copper concentrations.



Average & SEM

Time	PA 01 0	PA 01 0	PA 2397 0	PA 2397 0	PA 3516 0	PA 3516 0	PA 2520 0	PA 2520 0	PA 3790 0	PA 3790 0	PA 01 0.5	PA 01 0.5	PA 2397 0.5	PA 2397 0.5	PA 3516 0.5	PA 3516 0.5	PA 2520 0.5	PA 2520 0.5	PA 3790 0.5	PA 3790 0.5
0:00:00	0.04167239	0.0193342	0.03668292	0.0006237	0.05476976	0.013721	0.055393	0.000624	0.057888	0.000624	0.06410594	0	0.038535	0.001871	0.069095	0	0.056622	0.001247	0.058493	0.001871
0:10:00	0.04229608	0.01995789	0.03855397	0	0.05352239	0.013721	0.055393	0.000624	0.057888	0.000624	0.06472963	0.000624	0.039782	0.001871	0.067848	0	0.055998	0.001871	0.057245	0.000624
0:20:00	0.04229608	0.01995789	0.04167239	0.0006237	0.05352239	0.013721	0.056017	0	0.058512	0	0.06472963	0.000624	0.05288	0.012474	0.067848	0	0.056622	0.002495	0.057869	0.001247
0:30:00	0.04229608	0.01995789	0.04603818	0	0.05414607	0.0143447	0.059136	0.000624	0.057888	0.000624	0.065977	0.000624	0.055998	0.01185	0.068472	0.000624	0.05974	0.001871	0.058493	0.000624
0:40:00	0.04291976	0.02058157	0.05227502	0	0.05664081	0.0155921	0.063501	0	0.059136	0.000624	0.06784805	0	0.054127	0.003742	0.07159	0	0.064106	0.002495	0.060364	0
0:50:00	0.04416713	0.02058157	0.05913555	0.0006237	0.06038291	0.0168395	0.071609	0.000624	0.062254	0.001247	0.06847173	0.000624	0.064106	0.007484	0.077203	0.000624	0.073461	0.004366	0.063482	0.000624
1:00:00	0.04479081	0.02120526	0.06661975	0.0006237	0.06911449	0.0218289	0.082836	0.000624	0.067243	0.001247	0.0697191	0.000624	0.073461	0.008108	0.087806	0	0.087806	0.008732	0.068472	0.000624
1:10:00	0.04666186	0.02182894	0.0765987	0.0006237	0.07348028	0.0212053	0.096557	0.000624	0.077846	0.001871	0.0734612	0.000624	0.079074	0.004989	0.092172	0.000624	0.097785	0.004989	0.078451	0.001871
1:20:00	0.04978028	0.02245262	0.08782501	0.0006237	0.08221185	0.0224526	0.110278	0.001871	0.089696	0	0.07720331	0.000624	0.093419	0.004366	0.100903	0.001871	0.110882	0.003118	0.08843	0.000624
1:30:00	0.05414607	0.02432368	0.10029869	0.0006237	0.10528816	0.0318079	0.13772	0.006861	0.105288	0.000624	0.08344015	0.000624	0.115872	0.003118	0.120238	0.002495	0.13583	0.004366	0.101527	0.001247
1:40:00	0.05975923	0.0274421	0.1271171	0	0.13023552	0.0380447	0.16828	0	0.126493	0.001871	0.09279541	0.001247	0.139572	0.010603	0.16639	0.002495	0.165143	0.011226	0.121485	0.002495
1:50:00	0.06786712	0.03180789	0.15268815	0.0006237	0.16266709	0.0430342	0.190733	0.003742	0.155807	0	0.10464541	0.001871	0.155787	0.005613	0.195703	0.000624	0.193209	0.005613	0.155164	0.002495
2:00:00	0.08158817	0.03929209	0.17576446	0.0012474	0.19509866	0.0367974	0.211938	0.002495	0.184496	0.001247	0.11899014	0.003742	0.177616	0.001247	0.215037	0.001247	0.215037	0.007484	0.181982	0.000624
2:10:00	0.09156711	0.0430342	0.2131855	0.0037421	0.22191708	0.0299368	0.234391	0.003742	0.216304	0.001871	0.13458224	0.004366	0.193209	0.000624	0.238737	0	0.241856	0.001871	0.210672	0.001871
2:20:00	0.10528816	0.0467763	0.22940128	0.0012474	0.24249865	0.0218289	0.260585	0.004989	0.251854	0.001247	0.15765855	0.003742	0.210048	0	0.271169	0.004989	0.264309	0.005613	0.238114	0.000624
2:30:00	0.12399868	0.05301314	0.25809075	0.0012474	0.27617759	0.025571	0.286157	0.018087	0.271188	0.000624	0.18135854	0.007484	0.239361	0.005613	0.302977	0.015592	0.292374	0.006237	0.270545	0.003118
2:40:00	0.14083815	0.05363683	0.26994075	0.0018711	0.28553285	0.0212053	0.307985	0.004989	0.297383	0.004366	0.20194011	0.004366	0.26119	0.002495	0.326053	0.017463	0.321064	0.001247	0.297364	0.003742
2:50:00	0.1664092	0.05550788	0.27430653	0	0.32295389	0.013721	0.329191	0.007484	0.317964	0.003742	0.21503748	0.006237	0.278653	0.001247	0.349753	0.004989	0.334785	0.001247	0.324806	0.003742
3:00:00	0.19135656	0.04927104	0.28490916	0.0106026	0.33480388	0.0093553	0.354138	0.014968	0.340417	0.006237	0.23686642	0.008108	0.309837	0.007484	0.388422	0.004989	0.366593	0.005613	0.350377	0.008108
3:10:00	0.20881971	0.0430342	0.31235126	0.0006237	0.3616223	0.013721	0.362246	0.010603	0.357257	0.021829	0.25432957	0.005613	0.318569	0.004989	0.413993	0.000624	0.385303	0.001871	0.36784	0.005613
3:20:00	0.24000391	0.04178683	0.33106178	0.0006237	0.37409598	0.0162158	0.375967	0.025571	0.384075	0.002495	0.28426641	0.000624	0.341022	0.001247	0.44393	0.001871	0.405261	0.003118	0.397777	0.003118
3:30:00	0.25746707	0.03305525	0.35975125	0.0043658	0.40777492	0.0224526	0.400914	0.019334	0.413388	0.010603	0.30048219	0.009355	0.362851	0.008108	0.477608	0.001871	0.435822	0.006237	0.430832	0.001247
3:40:00	0.27368285	0.02307631	0.37347229	0.0031184	0.43833543	0.0268184	0.440206	0.012474	0.449562	0.005613	0.32044008	0.009355	0.385927	0.007484	0.508793	0.005613	0.450166	0.005613	0.463887	0.004366
3:50:00	0.29426442	0.02868947	0.38469861	0.0031184	0.46141174	0.0212053	0.460788	0.019334	0.468896	0.001247	0.34414007	0.004366	0.405885	0.011226	0.540601	0.006237	0.476985	0.004989	0.496319	0.006861
4:00:00	0.30174863	0.03243157	0.40153808	0.0012474	0.50319857	0.0280658	0.487606	0.009979	0.504446	0.008108	0.37781901	0.000624	0.423348	0.004989	0.568666	0.000624	0.506298	0.003118	0.533116	0.001247
4:10:00	0.31048021	0.03367894	0.41276439	0.0037421	0.54685645	0.0280658	0.528146	0.01185	0.535006	0.008732	0.40463742	0.008732	0.445177	0.008108	0.603593	0.000624	0.532493	0.01185	0.559935	0.000624
4:20:00	0.33230915	0.04178683	0.42586175	0.0043658	0.58552486	0.0305605	0.556835	0.005613	0.574299	0.006861	0.40588479	0.002495	0.457651	0.010603	0.644756	0.004366	0.546214	0.018087	0.588624	0.001871
4:30:00	0.36099861	0.02931315	0.43334596	0.0031184	0.62980643	0.0311842	0.595504	0.003118	0.616085	0.006237	0.425219	0.003118	0.468877	0.010603	0.68779	0.012474	0.571161	0.015592	0.616066	0.003118
4:40:00	0.38220387	0.03305525	0.4395828	0.0093553	0.67034589	0.0280658	0.629183	0.001871	0.652259	0.006237	0.46201635	0.006237	0.47948	0.016216	0.722716	0.009979	0.601098	0.009355	0.641637	0.003742
4:50:00	0.38469861	0.02681841	0.46265911	0.0012474	0.71400377	0.0318079	0.657249	0.004989	0.697788	0.009355	0.47760845	0.010603	0.493824	0.010603	0.763879	0.008732	0.622303	0.008108	0.671574	0.003742
5:00:00	0.41900123	0.03991578	0.4551749	0.0099789	0.76265113	0.0392921	0.687809	0.000624	0.735833	0.011226	0.4913295	0.000624	0.515653	0.003742	0.8013	0.006237	0.679682	0.043034	0.715232	0.001247

Table #6: Example of the final data transferred into GraphPad Prism 7. First column of paired mutant was the average, while the second column was the calculated SEM of their corresponding mutant.

Results

With the data being finalized, the graphs for all the growth curves were put together according to their experiment groups. The graphs are all organized under Appendix B. The graphs of the final data were then divided according to the independent compartmental regulation system that was previously mapped by Professor Argüello and his team.

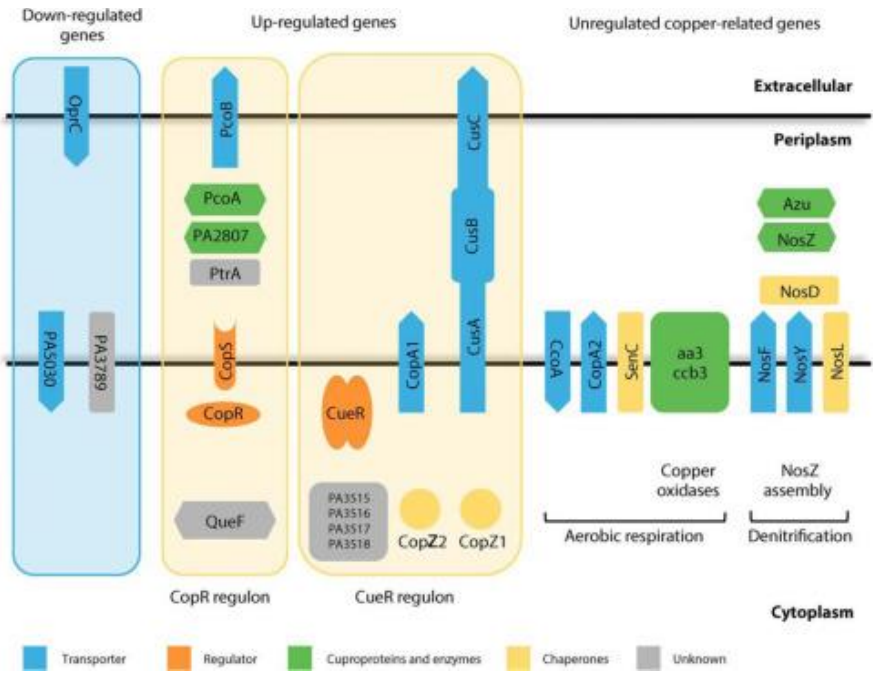


Figure #1: The independent compartmental regulation of *P. aeruginosa* showing the different mutant groups according to their function within the copper transport system. The system is divided into four sections: the down-regulated genes, the up-regulated genes (CopR regulon and CueR regulon), and the unregulated copper-related genes.

The figure shown above gives some of the genes that were used in this experiment. The full list of the mutants and their genes that were used in the experiment are in Appendix A. By comparing the growth curves of each mutant to the wild type, it was determined whether the mutant and its gene are phenotypes. Mutants that would not grow due to the copper concentrations were labeled are the phenotypes, while the mutants that did grow were considered unessential to the copper transport system. When a set of samples of a mutant shows a normal growth curve, the copper was correctly transported within the bacteria, allowing it to thrive in the well. Meanwhile, samples that would show no growth or slightly different growth curves to the wild type were considered phenotypes since the bacteria was not able to correctly transport copper in and out of itself due to the mutant gene.

P. aeruginosa **mutant**	**Phenotype** Yes/No	***P. aeruginosa*** **mutant**	**Phenotype** Yes/No
PA 3789	No	PA 1638	No
PA 3790	No	PA 3521	No
PA 2065	No	PA 3519	No
PA 2807	Yes	PA 3523	No
PA 2810	No	PA 3521	No
PA 4778	Yes	PA 3522	Yes
PA 2064	Yes	PA 3523	Yes
PA 2396	No	PA 1848	No
PA 2397	Yes		

Table #7: Results of the mutants' phenotype check. Divided by their grouping in figure #1: Down-regulated, CopR regulon, CueR regulon, and other regulation.

It was impertinent that the phenotypes were found since these would be the genes that are further studied in terms structure and mechanism within the gene complexes. Although these mutants were not studied and experiments during this time frame, the goal of the professor's lab will be to continue discovering more phenotypes and their limits. All other mutants and genes that were part of the experiment but not shown above or in the Appendices were considered non phenotypes or they were not even used in data collection time constrictions.

Conclusion & Discussion

As seen previously in the experiment, it is very difficult to find phenotypes since there are so many genes active in the copper transport system. Some genes are not functionally important to the system as others and finding the phenotypes this way is the best strategy to finding more and more mutants that are actually affected by their protein manipulation. Another reason why it might be difficult to find affected mutants since the growth curves are very peculiar and require to be done the same way every time. Any small human error such as miscalculations or measurements during the any of the steps of setting up the well plate. This makes finding phenotypes difficult since it is not always clear when there was human error, or just no growth due to the mutation and the copper interacting. Furthermore, phenotypes are important to find since they help map the functional and important genes that are a part of the copper transport system in the bacterium. They aid in eliminating the genes that are not so useful or crucial to the system. When comparing to the wild type, it makes sense that the phenotypes would have to be the bacterium that does not grow since the wild type will always grow until the 5 mM benchmark of copper. This should be because the gene mutation is shutting down a certain part of the system that either directly correlates with the in or out flow of copper inside of *P. aeruginosa*. If the system is missing a part in the influx of copper, then it will be unable to control the influx of copper; allowing more than should be accepted and ultimately killing the bacterium. However, if the system is mutated in the outflux of copper, then the bacteria will die from the inability to transport it out itself. This study was not limited to only transporters, so it is crucial to find out which of the other possible genes might be affected by mutations. Finally, it was clear that 6 genes (PA 2807, PA 4778, PA 2064, PA 2397, PA 3522, and PA 3523) were phenotypes under copper concentrations. Although PA 2807, PA 2064, and PA 4778 all have known functions and names, it is important that further research goes into PA 2397, PA 3522, and PA 3523 since they are part of the other section in the copper transport system; meaning they are unknown genes as far as function, structure, and mechanism goes. It is believed that there are many more phenotypes that have not been discovered, so there is plenty of more research to be done on the *Pseudomonas aeruginosa* copper transport system and its possible phenotype mutants.

Appendices

Appendix A (Mutants of *P. aeruginosa* and their possible known genes)

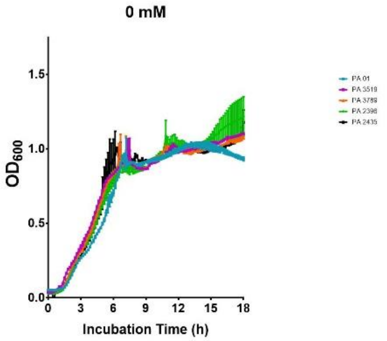
Redox Enzymes	
PA2065	pcoA
PA2807	cupredoxin -plastocyanin like
PA3392	NosZ
PA4922	Azu
Chaperones	
PA0114	SenC
PA3520	CopZ1
PA3574.1	CopZ2
Transporters	
PA1549	CopA2
PA1848	CcoA
PA2435	HmtA
PA2520	CusA
PA3789	

PA3790	OprC
PA3920	CopA1
PA5030	CuiT
Transcriptional regulators	
PA2809	CopR
PA2810	CopS
PA4778	CueR
Other Genes	
PA1638	Glutaminase
PA1890	Glutathione
PA2025	Glutathione
PA2064	pcoB
PA2396	Pyoverdine
PA2397	Pyoverdine
PA2398	Pyoverdine
PA3516	
PA3519	

PA3521	
PA3522	
PA3523	

Appendix B (graphs of all final data sheets)

Graphs of PA 01, PA 3519, PA 3789, PA 2396, PA 2435 (1-23-19)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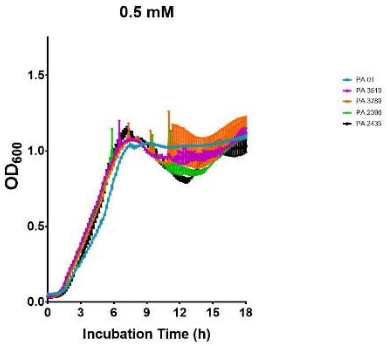
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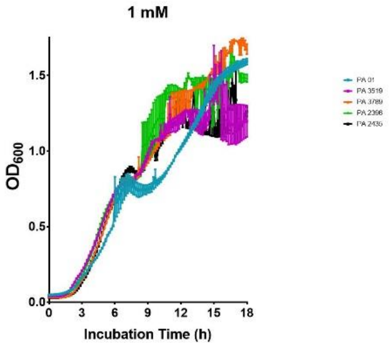
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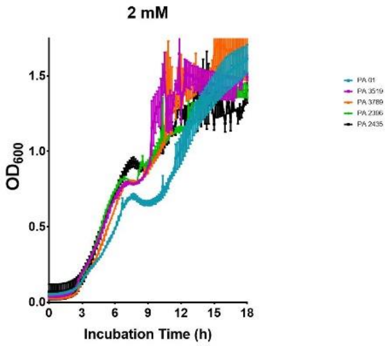
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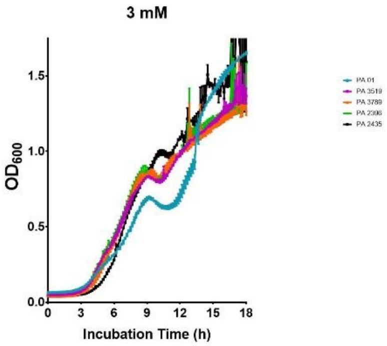
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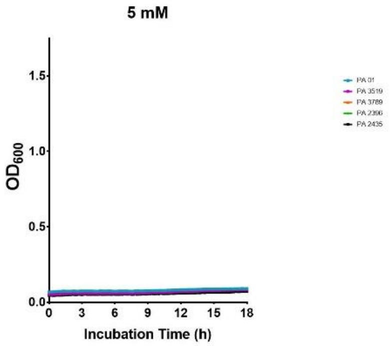
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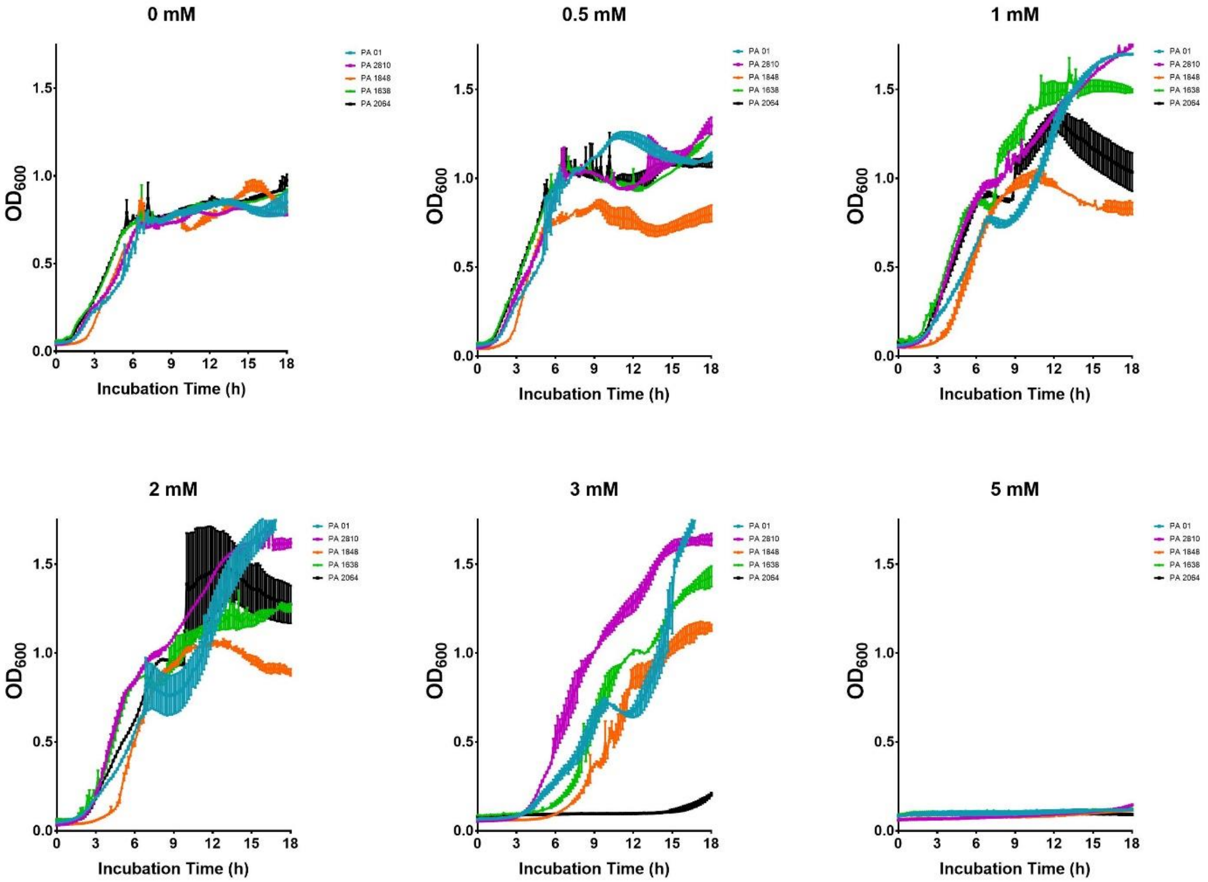
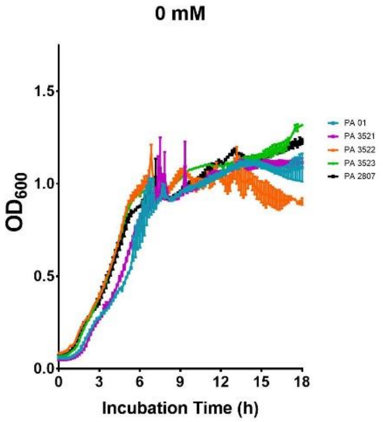
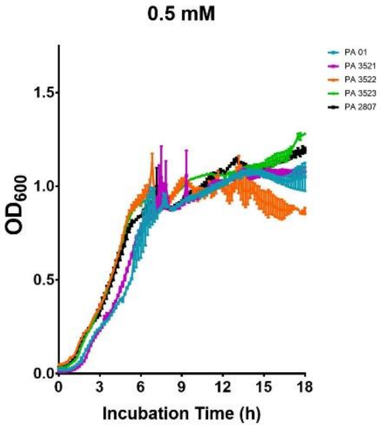
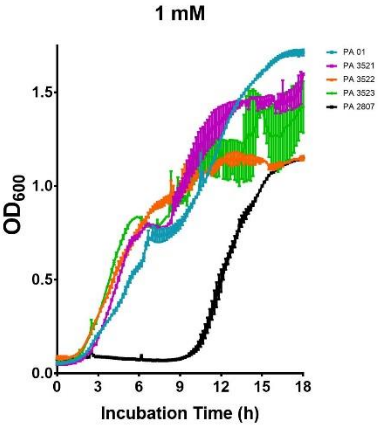
Graphs of PA 01, PA 2810, PA 1848, PA 1638, PA 2064 (1-24-19)

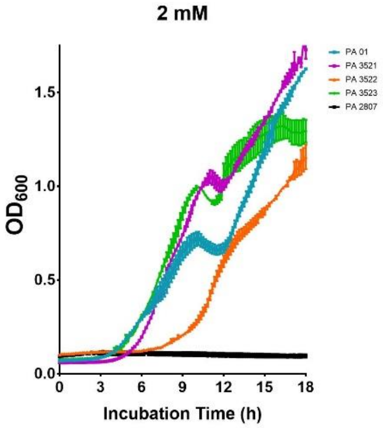


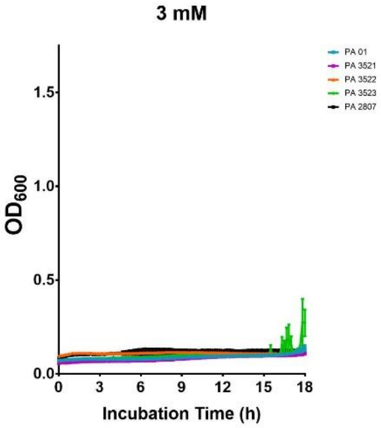
Graphs of PA 01, PA 3521, PA 3522, PA 3523, PA 2807 (1-30-19)

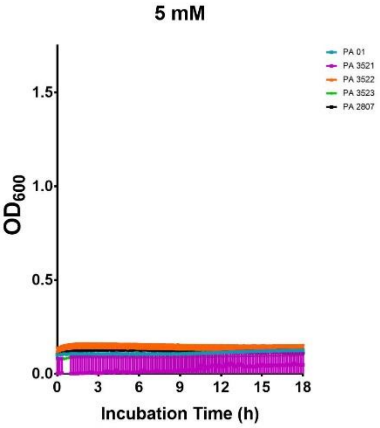




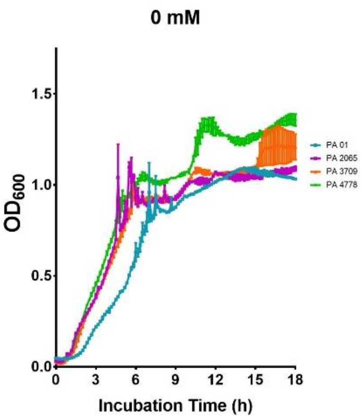








Graphs of PA 01, PA 2065, PA 3790, PA 4778 (1-31-19)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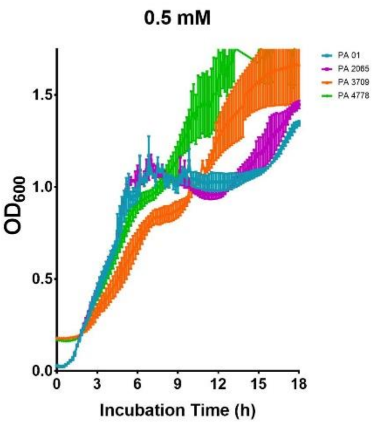
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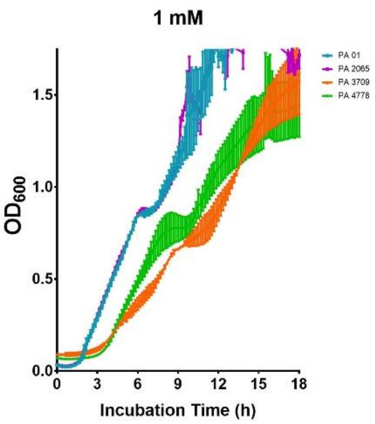
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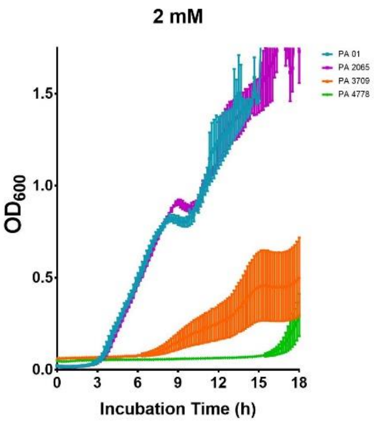
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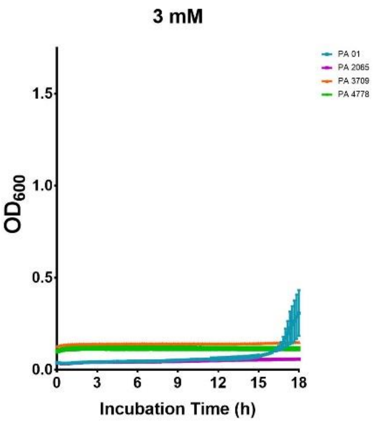
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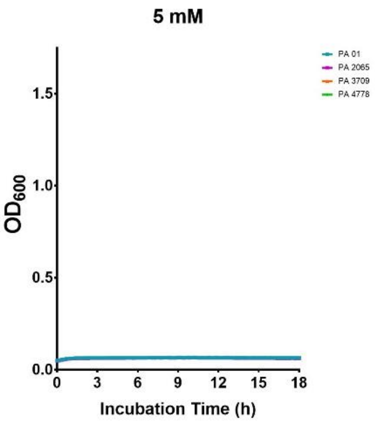
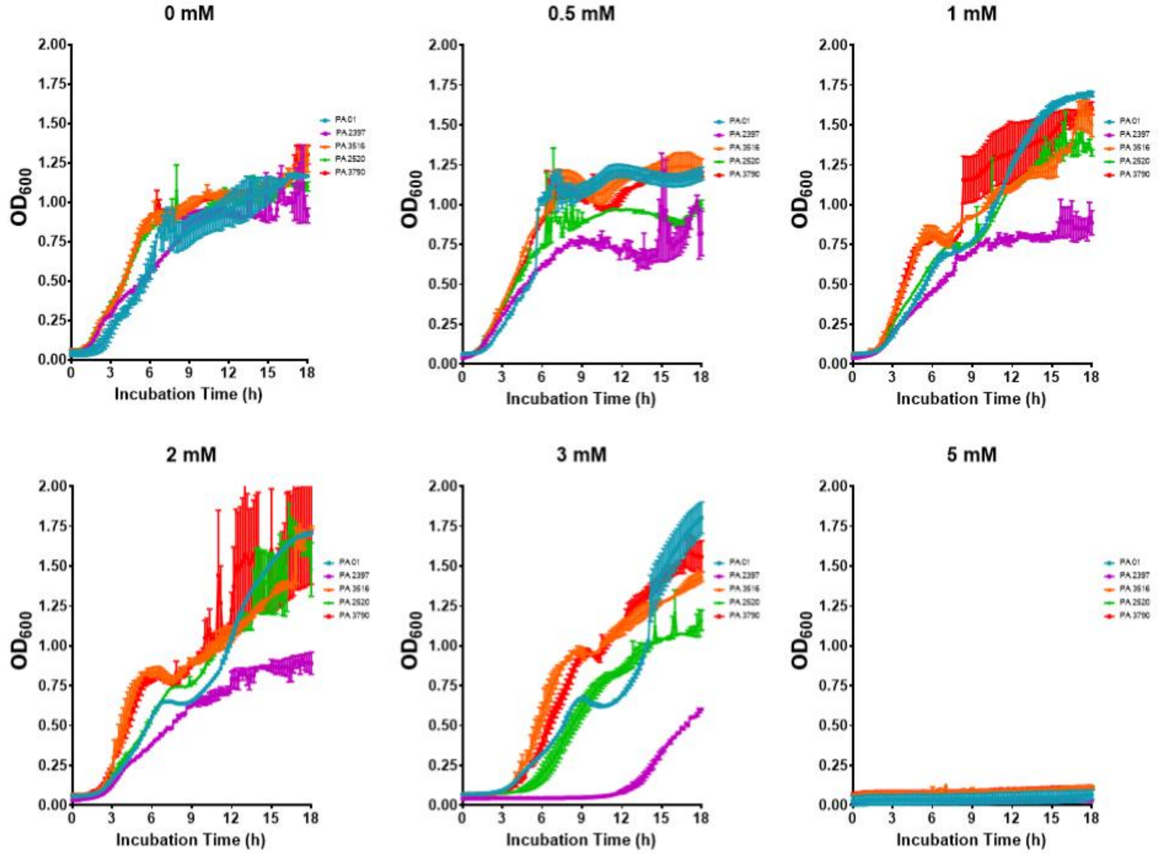

5 mM

Graph of PA 01, PA2397, PA 3516, PA 2520, PA 3790 (2-5-19)



Graph of PA 01, PA 2809, PA 2810, PA 2064, PA 2907 (2-22-19)