The Effects of Membrane Stress and Defects in Lipoprotein Maturation of *Acinetobacter Baylyi* Δlnt

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1. Introduction:

Throughout the past decade, frequency of antibiotic resistance (ABR) in the United States has increased drastically. Many pathogenic bacteria have evolved resistance to the main classes of antibiotics, and multidrug-resistant bacteria have caused untreatable infections. According to MacLean and Millan, "ABR already imposes substantial health and economic burdens, and the global annual cost of ABR could increase to 10 million deaths and US$100 trillion by 2050" (MacLean & Millan, 2019). ABR is frequently gained through spontaneous mutations, conjugation, or transduction. Antibiotic resistance is caused by bacteria in a population that have mutations which allow them to thrive even in the presence of a drug. These bacteria continue to grow and infect more humans because they cannot be killed by the original drug of choice. This vicious cycle of antibiotic resistance can lead to outbreaks of diseases and infections, which is a significant health threat to Americans.

Antibiotic resistant bacteria aren't just a problem for humans, its spreading to wildlife as well. More specifically, antibiotic resistant bacteria have been found in diseased wild dolphins. According to Galoustian, "Antibiotic resistance is one of the most significant risks to public health. As resistance increases, the probability of successfully treating infections caused by common pathogens decreases." Galoustian also explained the finding that there are currently dolphins in the ocean living with ABR. This is unhealthy for humans and the rest of the planet because it sets yet another platform where disease can spread.

Acinetobacter baumannii is a pathogen with a high rate of bacterial resistance formation. The pathogen usually targets the most vulnerable hospitalized patients who are critically ill with breaches in skin and airway protection. As reported from reviews dating back to the 1970s (Glew, 1977), hospital-acquired pneumonia is still the most common infection caused by this

sorting of lipoproteins (Harding, Hennon, & Feldman, 2017). Lipoproteins are synthesized as precursors in the cytoplasm and receive their acyl modifications as they are exported across the inner membrane. After this processing, they are sorted by the Lol transport system. The third tail of a lipoprotein enables it to be identified as a lipoprotein, and then it is assigned to a location in on the cell membrane (Lovullo, et al. 2015). As shown in Figure 1, Lnt is the final step before the lipoprotein is transported to the Lol trafficking system.

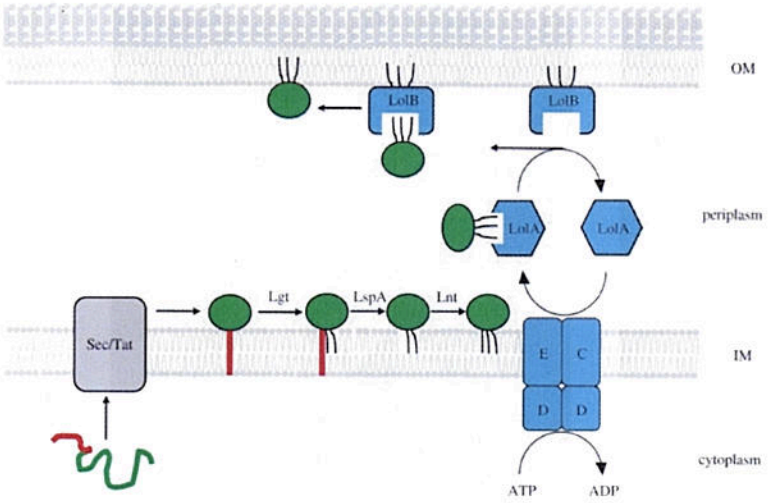

Figure 1: "Lol system processing shown above. Lipoprotein maturation and export pathway. Lipoprotein (green) is synthesized in the cytoplasm with the N-terminal SS (red) which targets it for translocation across the IM by the Sec or Tat translocon. The lipoprotein remains anchored in the IM and Lgt adds a diacylglyceryl moiety to the Cys residue. LspA cleaves the SS and Lnt adds another acyl chain to the newly formed N-terminus. The lipoprotein is then recognized by IM LolCDE complex which powers extraction of the lipoprotein from the IM using the energy of ATP. The lipoprotein is released to the periplasm in a complex with the chaperone LolA. LolA delivers lipoprotein to the OM acceptor protein LolB which inserts it in the inner leaflet of the OM. Empty LolA returns to LolCDE and is recycled." (Konovalova & Silhavy, 2015).

The Lnt enzyme adds the third acyl tale to the lipoprotein, allowing it to be identified by other receptors. So, without Lnt, it would be assumed that the identification would not occur, and the cell would not survive. This identification of being a lipoprotein is important for the cell because if the lipoproteins are not identified through their use of signals, then they will not move. The

efficient system for natural transformation and chromosomal incorporation of exogenous DNA. Studies that have used this microbe to focus on a wide array of problems, including gene duplication and amplification, horizontal gene transfer, bioreporters, and metabolic reconstruction. (Elliott & Neidle, 2011). Interesting results in these diverse areas highlight the utility of using *A.baylyi* in laboratory and industrial settings. In addition, *A. baylyi* is easily mutated and is gram-negative. The accessibility and safety of *A.baylyi* makes it an ideal bacteria to work with.

When Lnt is deleted from *A.baylyi*, the bacteria faces a great amount of stress. This stress occurs because the cell cannot use the traditional Lol pathway to transport lipoproteins. The stress that the bacteria will be put under will play a role in increasing the rate of suppressor mutations to occur. A suppressor mutation is a second mutation that masks the phenotypic effects of an earlier mutation, in this situation a suppressor mutation accounts for the absence of the Lnt gene. This experiment will aim to morphologically analyze suppressor mutations of A.baylyi Δlnt.

The gap in the research is actually the gap within the protein trafficking system. What unexplored pathway is enabling *A.baylyi* to thrive with the removal of the lnt gene? The unexplored gap in the protein trafficking system exists because the Lol system which was once thought to be the only system has been proven false by *A.baylyi Δlnt*. There is no other promising pathway that has been discovered which is aiding the traffic of lipoproteins in *A.baylyi.*

As a result of this study, a new class of antibiotics could be made to target the lnt gene. In addition, they could aid in the problem of antibiotic resistance. New antibiotics could be This is especially important since Lnt is a cell component that has not been the target of antibiotics, meaning it would not have mutated and developed as far as other target cell components have.

measured and recorded. Any additional suppressor mutants that were found in the zones-of-inhibition were struck out on LB plates.

Growth Curve:

Liquid cultures of the 12 strains were made 18-24 hours prior to experimentation. The optical density of each liquid culture was measured in a Biophotometer using a 1ml cuvette with 800ul of LB and 200ul of LB. This was done to normalize the concentration of each culture and to have about the same number of cells in each well of the spectrophotometer plate. The spectrophotometer was used to measure the optical density (OD600) of each strain of bacteria every 20 minutes over an 18-hour period.

EOP:

Two 96 well plates were used to dilute each of the 12 strains of bacteria from 10^0 to 10^{-5}, creating a ten-fold dilution moving from left to right. Two plates were used for each type of agar because all 12 strains could not fit onto 1 plate. Plate #1 used columns 1-8 and Plate #2 used columns 5-12. A replicator was then sterilized, flamed and dipped from the 96 well plate onto each agar plate. The types of agar plates that were used were MacConkey, Kan 25, Amp 100, and LB. These plates were left in the incubator overnight at 30°C.

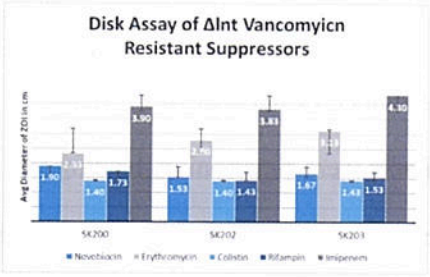

Figure 4: Kirby Bauer Disk Assay of ΔInt Vancomycin Resistant Suppressor Mutants. Graph shows inhibition zones for suppressors SK200, SK202 and SK203 with 5 different antibiotics. Error bars represent the standard deviation.

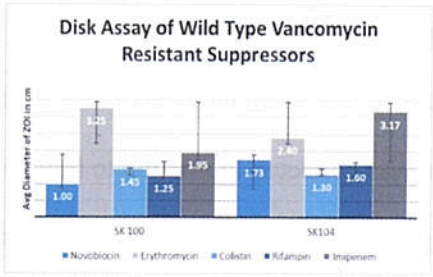

Figure 5: Kirby Bauer Disk Assay of WT Vancomycin Resistant Suppressor Mutants. Graph shows inhibition zones for suppressors SK100 and SK104 with 5 different antibiotics. Error bars represent the standard deviation.

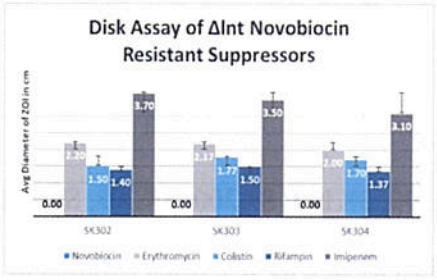

Figure 6: Kirby Bauer Disk Assay of ΔInt Novobiocin- Graph shows inhibition zones for suppressors SK302, SK303, and SK304 with 5 different antibiotics. Error bars represent the standard deviation.

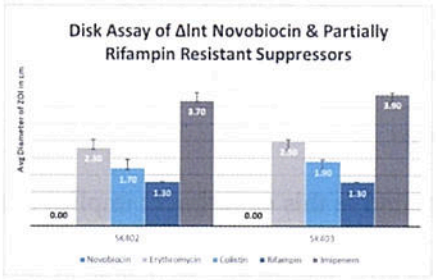

Figure 7: Kirby Bauer Disk Assay of ΔInt Novobiocin and Partially Rifampin Resistant Suppressor Mutants. Graph shows inhibition zones for suppressors SK402 and SK403 with 5 different antibiotics. Error bars represent the standard deviation.

As seen above, Figure 4 shows that in the Vancomycin resistant suppressors, Imipenem antibiotic has the largest ZOI. The disk assay of Wild Type Vancomycin Resistant Suppressors in Figure 5 revealed that SK100 and 104 were least resistant to Erythromycin. Suppressor mutants that were originally resistant to Vancomycin were found to be resistant to Novobiocin as well. This can be seen in Figures 6 and 7 as the ZOI for Novobiocin was measured to be 0cm on each plate.

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