Predicting the Biochemical Parameters of An Antibiotic Efflux Pump Using a Mathematical Model

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The Centers for Disease Control and Prevention report that at least 2 million people in the United States will become ill due to antibiotic resistant pathogens leading to 23,000 deaths each year. In order to circumvent these resistance mechanisms, it is essential to quantitatively understand how the function of the protein(s) involved relates directly to resistance. Integral membrane efflux pumps are known determinants of single-drug and multidrug-resistance in a wide variety of pathogenic organisms. These transporters are membrane proteins and characterization typically requires reconstitution in an artificial membrane. Subsequently, these important proteins are difficult to characterize by traditional in vitro studies.

My project aims to determine the physicochemical parameters of the efflux pump TetB utilizing molecular biology and mathematical modeling. TetB is composed of 12 transmembrane (TM) alpha helices and is found within the inner membrane of Gram-negative bacteria. This protein allows for the efflux of tetracycline (TET), doxycycline (DOX), and minocycline (MCN) from the cytoplasm into the periplasm. Tetracycline class of antibiotics are bacteriostatic and function by inhibiting protein translation. For cells grown in tetracyclines, the efflux mechanism of TetB allows for cytosolic antibiotic concentration to decrease and rate of protein translation to increase.

I have inserted a tet(B) expression system into the E. coli chromosome and have determined its growth profile under various concentrations of TET, MCN, and DOX using high-throughput 96-well plates. The growth rate profiles correlate with TetB pumping rates for each drug. TetB more readily pumps out TET compared with DOX and MCN and we observe that cells expressing TetB can grow at higher TET concentrations compared with DOX and MCN. We are currently working on understanding how efflux expression effects bacterial growth by testing ribosome binding site (RBS) sequences of varying strengths in our tet(B) expression system. The shapes of the growth rate profiles produced in the different drugs give insight into the physicochemical mechanism of TetB. We are currently working on building a mathematical model that can simulate these growth profiles and predict efflux pump physicochemical parameters. Future works are geared toward modeling more complex efflux pump such as the tripartite pumps which traverse both bacterial membranes and cause multi-drug resistance. Collectively, this project aims to build an in vivo system will allow for the characterization of a variety of efflux pumps without the arduous tasks of protein purification and efflux pump reconstitution.

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