Novel combinations of efflux pumps improve fitness landscape of *E. coli* under biofuel stress

William J. Turner, Mary J. Dunlop

Recent advances in synthetic biology have enabled the construction of non-native metabolic pathways for production of next-generation biofuels in microbes. One such biofuel is the jet-fuel precursor α-pinene, which can be processed into high-energy pinene dimers. However, accumulation of toxic biofuels in the growth medium limits the possible fuel yield. Overexpression of transporter proteins such as efflux pumps can increase tolerance to biofuels by pumping them out of the cell, thus improving fuel yields. However, too many active efflux pumps can compromise the cell as well, creating a trade-off between biofuel toxicity and pump toxicity. Previous research has suggested that certain combinations of efflux pumps can confer additional tolerance compared to the individual pumps themselves. However, the functional form of the combination of the tolerance provided by each pump and the toxicity due to their simultaneous activity is unknown. Using differential equations, we have developed a growth model incorporating the trade-offs between toxicity of α-pinene and efflux pump activity to describe the dynamics of bacterial growth under these conditions. By analyzing biofuel toxicity and the effects of each efflux pump independently through a series of experiments and mathematical models, we propose a functional form for their combined effect on growth rate. The maximum growth rate as a function of promoter activity and biofuel concentration is modeled and compared to experimental data with the ultimate goal of guiding design of tolerance mechanisms to improve overall biofuel yield.