

## ABSTRACT

**DISSERTATION/THESIS/RESEARCH PAPER/CREATIVE PROJECT:** Optimization of a  $\beta$ -galactosidase Fluorescence Reporter Assay for Determination of ykkCD Riboswitch Regulation in *B. subtilis*

**STUDENT:** Steven V. Trick

**DEGREE:** Master of Arts

**COLLEGE:** Sciences and Humanities

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Today, 70% of all bacterial strains are resistant to at least one antibiotic. Bacteria have become resistant to antibiotics through several methods, including mutations in their genome. Antibiotic resistance continues to grow based on several factors. These include over-prescription of antibiotics by physicians, non-completion of prescribed antibiotic treatments by patients and use of antibiotics in animals as growth enhancers by the food industry. Other causes are increased international travel and poor hospital hygiene. Three methods have been discovered that bacteria can employ to resist antibiotics. Bacteria can degrade, modify, or through the use of an efflux pump, pump the antibiotic out of cells. The focus of this study is on the third mechanism, the triggering of production of these efflux pumps. Efflux pumps are most commonly regulated by transcription factors. However, RNA sensors may also regulate them. A riboswitch is one example of an RNA sensor capable of changing gene expression patterns in response to internal and external signals. It is a highly structured region of the mRNA that specifically binds to a small target, often the product of the gene to be regulated. Once the concentration of this target molecule reaches a threshold, it binds to the riboswitch which can either turn on or turn off expression of this metabolite-producing gene. The *ykkCD* RNA is a small riboswitch that is suspected to act as an antibiotic sensor in *B. subtilis*, and is encoded immediately adjacent to a multidrug-resistant (MDR) *ykkCD* efflux pump. It has already been demonstrated that the *ykkCD* riboswitch sensor specifically recognizes tetracycline. This binding event is expected to trigger production of the *ykkCD* efflux pump causing the removal of the toxic tetracycline from the cell. If tetracycline or its derivatives interact with the putative *ykkCD* riboswitch, an increase in the *ykkCD* pump expression levels *in vivo* would be expected when exposed to tetracycline or its derivatives. Two

different procedures are necessary to determine if pump expression levels increase when exposed to tetracycline. First, in order to evaluate the amount of *ykkCD* pump mRNA, *B. subtilis* cells were grown with and without tetracycline or its derivatives, and *ykkCD* pump mRNA levels were quantified using quantitative real-time PCR. Second, the amount of *ykkCD* pump protein produced in the presence of tetracycline and its derivatives was quantified with a fluorescence assay using a *ykkCD* RNA- $\beta$ -galactosidase reporter gene construct. In this construct the *ykkCD* pump genes were replaced with the  $\beta$ -galactosidase gene. In the case of antibiotics that retain binding, an increase in pump mRNA levels and high  $\beta$ -galactosidase activity would be expected. Together these two results shed light on the *in vivo* antibiotic specificity of the *ykkCD* RNA. The focus of this paper is the optimization of a  $\beta$ -galactosidase fluorescence reporter assay to study the regulation of the *ykkCD* efflux pump by tetracycline and its derivatives in *B. subtilis*. Using this procedure and the quantitative real-time PCR, preliminary results indicate that when *B. subtilis* cells containing the *ykkCD-lacZ* construct were grown with the antibiotic tetracycline  $\beta$ -galactosidase activity increased, indicating upregulation of gene expression. This would seem to indicate that the presence of tetracycline turns on the gene for the production of a pump for the removal of itself.