# Impacts of genotoxic agents on rates of ribosomal mutations in the 30S ribosomal rpsE gene

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## Introduction

Antibiotics are critical to modern medicine and allow clinicians to combat pathogenic bacteria. Resistance to these important drugs is hindering our ability to treat many common infections. A single nucleotide change can alter the DNA in a way that provides bacteria with antibiotic resistance. In *Bacillus subtilis,* the *rpsE* gene is responsible for encoding a portion of the 30S ribosomal subunit. Single nucleotide mutations in the *rpsE* gene provide protection from the effects of the ribosome targeting antibiotic spectinomycin. To understand the role of genotoxic stress in the development of resistance conferring mutations, we aim to study the impacts of genotoxic stress on mutation in the *rpsE* gene of the bacteria *Bacillus subtilis*. We will define whether treatment with specific genotoxic agents results in a bacterial stress response, leading to an increase in the frequency of *rpsE* mutants resistant to spectinomycin. Sequencing a library of these mutants using the Illumina next generation sequencing platform will allow us to characterize how various genotoxic agents impact the prevalence of mutations at different nucleotide positions in the *rpsE* gene.

## Background

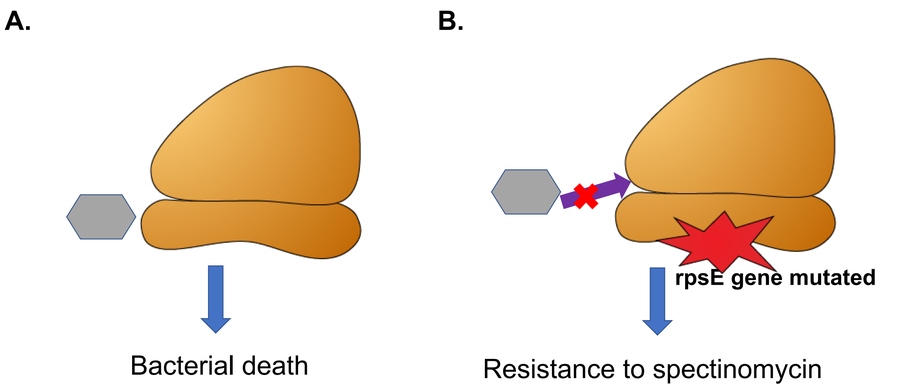


**Figure 1. Spectinomycin is a bactericidal antibiotic that inhibits protein synthesis in bacteria and its site of action is the 30S ribosomal subunit. The wild type ribosome of *B. subtilis* is susceptible to spectinomycin (A), while a single nucleotide mutation in the *rpsE* gene leads to spectinomycin resistance (B).**

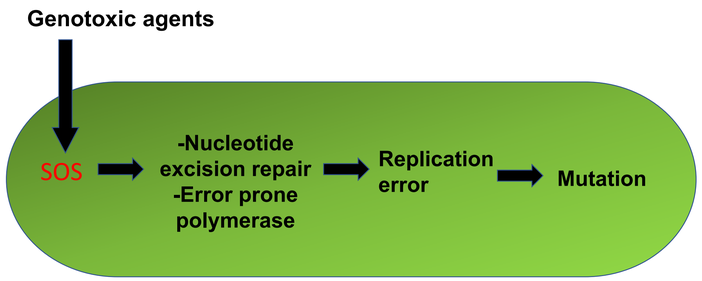


**Figure 2. Genotoxic agents induce mutations through DNA damage and activation of error prone polymerases and nucleotide excision repair mechanisms during the SOS response.**

## Methods

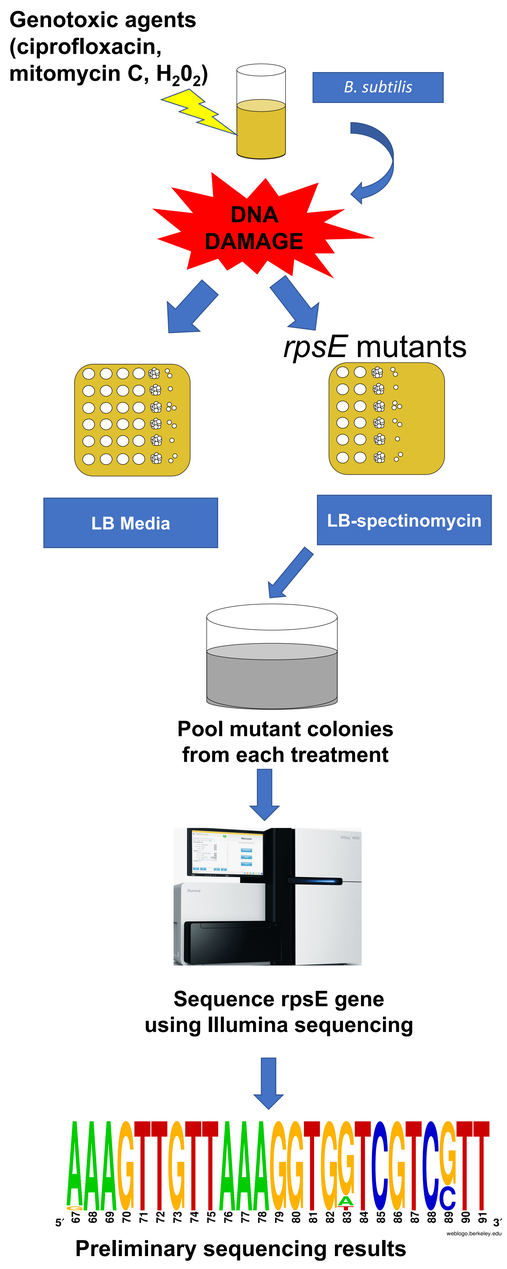


**Figure 3. Pipeline to examine genotoxic stress-induced mutations in the *rpsE* gene utilizing a high throughput method of microbiological culturing assays and Nextgen sequencing.**

## Results

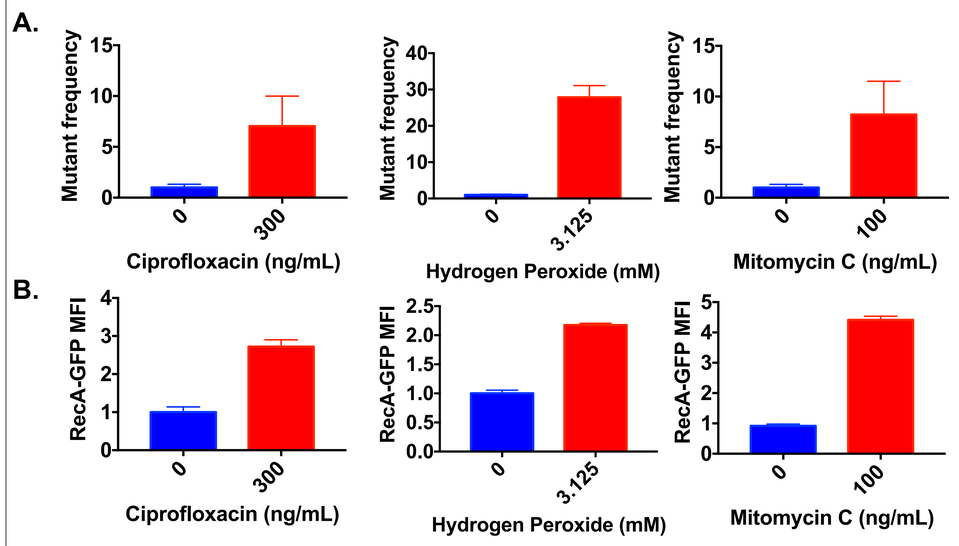


**Figure 4. Fold change of *rpsE* mutant colony formation (A) and RecA-GFP fluorescent intensity (B) after exposure to genotoxic agents (ciprofloxacin, hydrogen peroxide, and mitomycin C).**

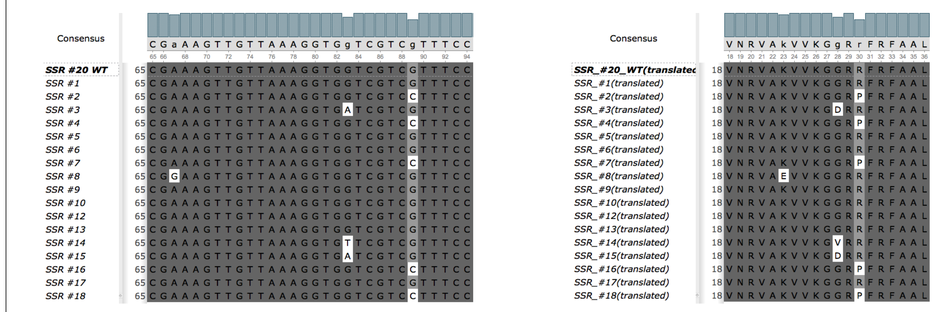


**Figure 5. Nucleotide (A) and amino acid (B) sequences of the *rpsE* gene in 17 mutant colonies generated by treatment with mitomycin C.**

## Conclusions and Future Directions

- Genotoxic agents activate SOS response and increase frequency of rpsE mutants.
- Preliminary sequencing shows that there are three nucleotide sites that show mutations.
- Categorize mutants by specific genotoxic agents utilizing Illumina Sequencing
- Explore other genotoxic agents and their impacts (ampicillin, nalidixic acid, UV, heavy metals, etc.)

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