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Mutations to antibiotic resistance during stringent response in B. subtilis

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The relA gene in *Bacillus subtilis* controls a variety of factors during the stringent response which is a response to starvation of amino acids. The stringent response inhibits DNA synthesis and transcription of genes of tRNA, rRNA, and ribosomal proteins and promotes synthesis of the required amino acids. The objective of my project is to determine if a strain of *B. subtilis* that has a knockout mutation for the relA gene will accumulate a higher number of mutations that confer resistance to antibiotics that inhibit translation. It is proposed that because the relA gene inhibits transcription of ribosomal proteins, a strain lacking this gene will transcribe more rRNA and ribosomal proteins and promote the generation of mutations that target the translation process.
Mutations to Antibiotic Resistance During Stringent Response in *B. subtilis*

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Abstract

The stringent response is a global regulatory control mechanism that is activated by amino acid starvation. The relA gene in *B. subtilis* controls a variety of factors during the stringent response which include the inhibition of DNA synthesis and inhibition of transcription of genes of RNA, RNAI, RNAII, and ribosomal proteins. The relA gene also promotes synthesis of the lacking amino acids. We are investigating if a strain of *B. subtilis* that lacks a functional relA gene will accumulate a higher number of mutations that confer resistance to antibiotics that inhibit translation. It is proposed that because the relA gene inhibits transcription of ribosomal proteins, a strain lacking this gene will transcribe more RNAII and ribosomal proteins and thus promote the generation of mutations that target the translation process.

Hypothesis

It has been observed that when a *B. subtilis* relA mutant is placed under aminoacid starvation it will produce less reversion mutations for amino acid prototrophic markers than the wild-type. This is most likely due to a decreased transcription for some of the genes involved in the synthesis of amino acids. Our hypothesis is that when we challenge a relA mutant with rifampicin, a transcription inhibiting antibiotic, the same number of mutations to resistance will occur when compared to the number of mutations in the wild-type *B. subtilis* strain. It is also hypothesized that when we challenge each strain with a translation inhibiting antibiotic, the number of resistance mutations in the relA mutant strain will be noticeably higher than that of the isogenic wild-type. This assumption is based on the observation that the relA mutant strain produces more RNAI due to a lack of RNAII transcription inhibition that would normally occur under the stringent response.

Methods

Construction of isogenic mutant relA strain. We used the *B. Subtilis* strain R1690 obtained from Kawamura lab which had relA replaced by an erythromycin cassette. The DNA was extracted from R16900 and transformed onto YB955. The mutant was checked with PCR for existence of the relA gene. No fragments appeared in the R16900 or YB955 relA strain using electrophoresis, while the wild-type YB955 had a fragment that was 2.2 kb, indicating that the relA gene was absent in the mutant. We then used the newly transformed strain onto media containing erythromycin to eliminate any background non-mutant colonies.

Determination of minimal inhibitory concentration: We plated YB955 onto Erlenmeyer flasks containing different concentrations of the antibiotic, which inhibits the beta-subunit of RNA polymerase, to determine the minimal inhibitory concentration.

Results

PCR Verification of relA mutant Colonies.

![PCR Verification of relA mutant Colonies](image)

Figure 2. Gel of PCR. Lane 1-3 kb ladder 2- YB955 3- YB955 1/10 dilution 4- YB955 Mutant 5- YB955 mutant 6- R16900 7- R16900. Strains containing relA gene should have a band at 2.2 kb, whereas strains with the gene knockout should not contain any bands.

Minimal Inhibitory Concentration

![Minimal Inhibitory Concentration](image)

Figure 3. Growth (red bars) stopped at 1.25 x 10^6. Resistant colonies grew from 2.5 and above. The correct concentration of ampicillin used will be 2 x 10^7. This concentration not allow growth but will allow mutations to resist it to occur.

Conclusions

The experiment has been set up with the correct parameters and the actual mutagenesis portion of the experiment is now in progress.

Future Directions

After performing this experiment with rifampicin we will then do the same method with tetracycline, a bacteriostatic antibiotic, as well as chloramphenicol, a bacteriocidal antibiotic.

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