Aug 6th, 9:30 AM - 12:00 PM

Mutations to antibiotic resistance during stringent response in B. subtilis

Chad Hansen
University of Nevada, Las Vegas

Ronald E. Yasbin
University of Nevada, Las Vegas

Eduardo A. Robleto
University of Nevada, Las Vegas

Repository Citation
http://digitalscholarship.unlv.edu/cs_urop/2009/aug6/16

This Event is brought to you for free and open access by the Undergraduate Research at Digital Scholarship@UNLV. It has been accepted for inclusion in Undergraduate Research Opportunities Program (UROP) by an authorized administrator of Digital Scholarship@UNLV. For more information, please contact digitalscholarship@unlv.edu.
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

Chad Hansen, Ronald E. Yasbin, and Eduardo A. Robleto
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV

Abstract

The stringent response is a global regulatory control mechanism that is activated by amino acid starvation. The relA gene in Bacillus 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, RNA, 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 in genes coding for amino acids that inhibit translation. It is proposed that because the relA gene inhibits transcription of ribosomal proteins, a strain lacking this gene will transcribe more RNA 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 amino acid starvation, it will produce less reversion mutations for amino acid prototrophy 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 YH955 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 RNA due to a lack of RNA transcription inhibition that would normally occur under the stringent response.

Methods

Construction of isogenic mutant relA strain. We used the B. Subtilis strain RIK500 obtained from Kawasaki lab which had relA replaced by an erythromycin cassette. The DNA was extracted from RIK500 and transformed onto YH955. The mutant was checked with PCR for existence of the relA gene. No fragments appeared in the RIK500 or YH955 relA/cassette using electrophoresis, while the wild-type YH955 had a fragment that was 2.2 kb, indicating that the relA gene was absent in the mutant. We then used the YH955 to transform the newly transformed strain onto media containing erythromycin to eliminate any background non-mutant cells.

Determination of Minimal inhibitory concentration: We plated YH955 onto rifampicin plates containing different concentrations of the antibiotic, which inhibits the beta subunit of DNA polymerase, to determine the minimal inhibitory concentration. The experiment was set up with the correct parameters and the actual mutagenesis portion of the experiment is now in progress.

Results

PCR Verification of relA mutant Colonies.

![Minimal Inhibitory Concentration](image)

Conclusions

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

Future Directions

References

Acknowledgement

I would like to thank Katherine Oba for her guidance and assistance on this project. I would also like to thank Holly Martin, Alessio Lurid, Mary Girard, and Carmen Yelin for their assistance on this project. This project is supported by grants.