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

The Role of rpoE in stationary phase mutagenesis in Bacillus

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Repository Citation
Undergraduate Research Opportunities Program (UROP). 13.  

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Stationary phase mutagenesis is a phenomenon whereby random mutations are generated in non-dividing cells. In order to understand how these mutations arise, we use Bacillus subtilis, a gram positive rod-shaped model organism. It is hypothesized that increased transcription promotes stationary phase mutagenesis in this organism. We therefore examined the role of rpoE, a gene that encodes RNA polymerase δ subunit and proposed to influence efficiency of transcription. To this end, we will first generate a strain bearing a deletion in the rpoE gene. In order to determine if this gene is important for mutagenesis, we will examine the accumulation of mutations in this strain compared to the wild type by scoring for reversion to auxotrophy. If rpoE is significant in this process, we will expect a difference between the accumulation of mutations in the mutant strain and wild type. This project is a step towards understanding stationary phase mutagenesis, a process that has implications in evolution, drug resistance and cancer formation.
The Role of \textit{rpoE} in Stationary Phase Mutagenesis in Bacillus

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Abstract

Stationary phase mutagenesis is a phenomenon whereby random mutations are generated in non-dividing cells. In order to understand how these mutations arise, we use B. subtilis, a Gram positive rod-shaped model organism. Transcription is one of the major processes hypothesized to drive stationary phase mutagenesis in this organism. We therefore examined the role of \textit{rpoE}, a gene that encodes for an RNA polymerase delta subunit which is up regulated during stationary phase. To this end, we will first generate a strain bearing a deletion in the \textit{rpoE} gene. In order to determine if this gene is important for mutagenesis, we will examine the rate of mutations in this strain compared to wild type by scoring for reversions to auxotrophy. If \textit{rpoE} is significant in this process, we will expect a difference between the rate of mutations in the mutant strain and wild type. This project is a step towards understanding stationary phase mutagenesis, a process that has implications in evolution, drug resistance and cancer formation.

Methods

1) Transformed YB955 with genomic DNA from an \textit{rpoE} deletion mutant in order to obtain isogenic wild type and mutant strains.
2) Inactivation of the \textit{rpoE} gene was verified using FCR.
3) Stationary phase mutagenesis assay was performed on both strains – cells are starved for amino acids for up to 9 days and revertants are scored.

![Bacillustest culture grows to T_{	ext{max}}](image)

A) Count revertants for 9 days incubation at 37\degree C
B) Survival rate (non-revertants)
- Remove one agar plug per plate
- Mix with plugs from Spizizen Salts
- Serial dilute
- Spread on plate

![Graphs](image)

Conclusion

It seems to be that \textit{rpoE} gene has no significant effect on stationary phase mutagenesis.

Acknowledgments

I would like to thank Eduardo Robleto and Kathernina Ona for their guidance and assistance on this project. I would also like to thank Robleto Lab for their advice on this project. Also, I would like to thank NSF funding and Dr. Narwin Dahal advising.

Hypothesis

We hypothesized \textit{rpoE} plays a role in stationary phase mutagenesis in \textit{Bacillus subtilis}. To test this hypothesis, I will knock out the \textit{rpoE} gene and compare to a wild type strain during stationary phase mutagenesis.

Figure 1. Stationary Phase Mutagenesis Assay

![PCR gel](image)

Figure 2. Verification of knockout via PCR