

The Role of *rpoE* in Stationary Phase Mutagenesis in *Bacillus Subtilis*



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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 *Bacillus 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 *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 *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 reversion to auxotrophy. If *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.

Introduction

- Stationary phase mutagenesis (SPM), also known as stress-induced or adaptive mutagenesis, is defined as the accumulation of mutations during conditions of no net growth or conditions of stress.
- Understanding SPM is important because it has been implicated in antibiotic resistance, evasion of the immune system and evolution. Also, this phenomenon has been implicated in formation of tumors.
- Our laboratory has shown evidence that suggest that accumulation of mutations in a gene depends on its level of transcription.
- An alternate RNA polymerase subunit, encoded by *rpoE*, has been shown to be active during stationary phase and affect the overall stability of the RNA polymerase; however, its role in stationary phase mutagenesis has not been examined [1].

Hypothesis

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

Methods

- 1) Transformed YB955 with genomic DNA from an *rpoE* deletion mutant in order to obtain isogenic wild type and mutant strains.
- 2) Inactivation of the *rpoE* gene was verified using PCR.
- 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.

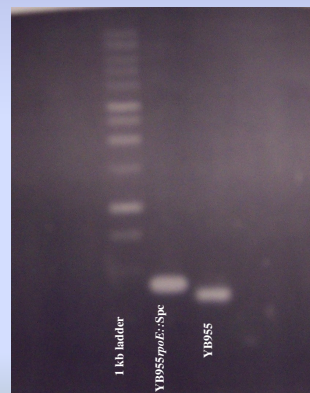
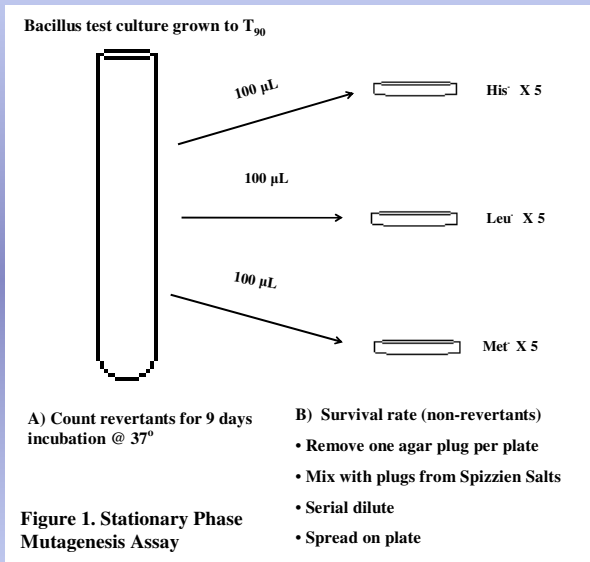
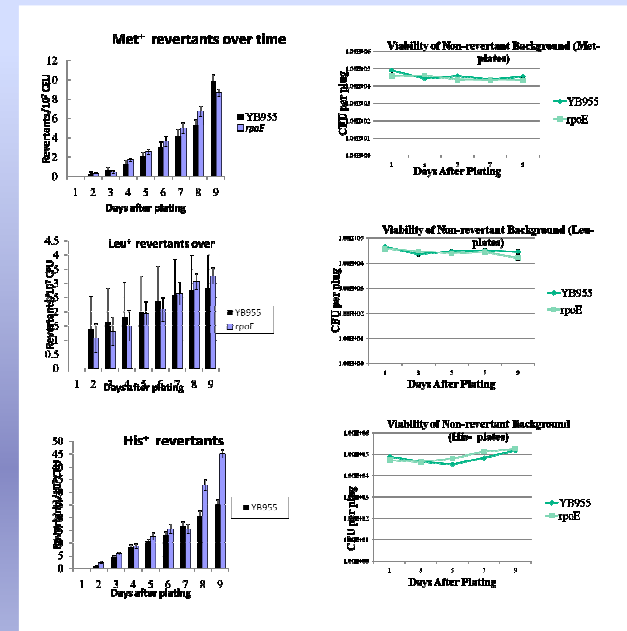


Figure 2. Verification of knockout via PCR

Results



Conclusion

It seems to be that *rpoE* gene has no significant effect on stationary phase mutagenesis.

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