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Stationary phase mutagenesis in Bacillus subtilis: The interaction between transcription and error-prone replication in conditions of stress

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While under conditions of stress, non-dividing cells may acquire beneficial mutations. This is referred to as stationary phase mutagenesis, or adaptive mutagenesis. Previous research has shown that actively transcribed genes and those under selective pressure are prone to mutations that confer escape from non-dividing conditions. Accordingly, strains lacking transcription factors have shown a drastically lower number of mutations that confer escape while under amino acid starvation than those observed in the wildtype background. Also, error-prone DNA polymerases are known to be active in cells under stress and it has been shown that strains lacking an error-prone DNA polymerase display reduced levels of stationary phase mutagenesis. It is possible to speculate that when active transcription stalls, perhaps due to pre-mutagenic lesions in the template DNA strand, error-prone polymerases are recruited to the site of stalled transcription as part of DNA repair processes. This interaction between transcription and DNA repair is likely to bias the accumulation of mutations at highly transcribed loci. This model may be tested with strains carrying deficiencies in Mfd (transcription factor), YqjH (error-prone DNA polymerase), or both. We expect the double-knockout strain to show a similar level of mutagenesis to those observed in strains carrying only one deficiency, and lower levels compared to those in the wildtype. Alternatively, if these factors influence mutation separately, a double-knockout should show even lower accumulation of adaptive mutants than either the Mfd⁻ or YqjH⁻. We are currently constructing the double-knockout strain in *Bacillus subtilis*. 
Stationary Phase Mutagenesis in Bacillus subtilis: the interaction between transcription and error-prone replication in conditions of stress

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Abstract:
While under conditions of stress, non-dividing cells may randomly acquire beneficial mutations. This is referred to as stationary phase mutagenesis, or adaptive mutagenesis. Previous research has shown that stationary phase mutagenesis occurs under nutrient starvation and stress conditions. The current study aimed to investigate the role of transcription and error-prone replication in the acquisition of beneficial mutations in stationary phase Bacillus subtilis. The study was designed to determine if (i) YgbH (polV) and mfd interact either within the same pathway, or in an additive fashion, to influence stationary phase mutagenesis, and (ii) if single and double mutations on the stationary phase mutagenesis assay in order to construct the double mutant, we introduced a disrupted mfd allele into the B. subtilis strain containing a defective YgbH allele.

Null hypothesis:
Previous research has shown that the process of transcription in genes under selection facilitates the accumulation of mutations that confer escape from non-dividing conditions. This specific interaction between transcription and DNA repair is likely to introduce mutations that confer escape from non-growing conditions at transcriptional level.

Research Methods:

Strategy and Aim: (i) To determine if YgbH (polV) and mfd interact either within the same pathway, or in an additive fashion, to influence stationary phase mutagenesis, and (ii) to examine the effects of single and double mutants on the stationary phase mutagenesis assay in order to construct the double mutant, we will introduce a disrupted mfd allele into the B. subtilis strain containing a defective YgbH allele.

Double Mutant Strain Construction:
A 600 bp fragment was PCR amplified out of the wild type strain using primers with ScIDMS1R and BMS1L. This segment was then digested and ligated into pUTMIN4 (see Fig 3)

![Figure 3: Double Mutant Strain Construction](image)

Conclusions & Future Directions:

- The pUTMIN4::mfdC::erm$^R$ plasmid was properly transformed into E. coli.
- The double mutant was constructed by transforming pUTMIN4::mfdC::erm$^R$ into YB55::ygbH::tet$^R$.
- This double mutant strain will then be used in our previously described stationary phase mutagenesis assay, along with the isogenic wildtype strain (YB955), the strain with the disrupted ygbH allele, and the strain with the defective mfd allele.

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