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

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Stationary Phase Mutagenesis in *Bacillus subtilis*: the interaction between transcription and error-prone replication in conditions of stress

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- strain. 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 involves changes in gene expression that lead to increased mutation rates. This study aimed to explore the role of transcriptional and translational changes in the context of adaptive mutagenesis. The hypothesis is that adaptive mutagenesis occurs through the rewiring of transcriptional networks to favor the accumulation of beneficial mutations. The research methods involved the use of *Bacillus subtilis* as a model organism to investigate the genetic and molecular mechanisms underlying adaptive mutagenesis.

**Research Methods:**

- **Strategy and Aim:** To determine if the *ydhG* (polY) and *mfd* genes interact either within the same pathway, or in an additive fashion, to influence stationary phase mutagenesis.
- **Experiment:**
  - *ydhG* and *mfd* were overexpressed in *B. subtilis* to study the effect on mutation rates.
  - The *ydhG* and *mfd* genes were knocked out to assess the impact on stationary phase mutagenesis.
  - The growth rate and viability of the *B. subtilis* cultures were monitored over time.

**Double Mutant Construction:**

- A 500 bp fragment was amplified using PCR and cloned into the *B. subtilis* plasmid pMS1004 (see Fig. 3).

**Conclusions & Future Directions:**

- The pMS1004 plasmid was properly transformed into *E. coli*.
- The double mutant was constructed by transforming pMS1004 into *B. subtilis*.
- This double mutant strain will then be used in our previously described stationary phase mutagenesis assay, along with the isogenic wildtype strain (YB955) to test the strain with the disrupted *ydhG* and the strain with the defective *mfd* allele.

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**References:**