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 conditions of stress. In this paper, we describe a model system for studying the interaction between transcription and error-prone replication in stationary phase conditions.

Background:
Stationary phase mutagenesis, or adaptive mutagenesis, occurs when non-dividing cells randomly acquire beneficial mutations while under stress.

- In E. coli, stress-induced mutations may arise via the differentiation of a hypermutable subpopulation of cells. These cells exhibit hypermutation in time and in genotypic space (Sahai, 2002).
- In B. subtilis, the gyh, ygh and mfi genes encode an error-prone DNA polymerase and mfi encodes a transcription elongation factor. Previous research has shown that strains deficient in these genes show a reduced number of mutations that confer escape from amino and starvation (Kim et al., 2001; Ross et al., 2006). See figures 1 and 2.

Hypothesis:
Previous research has shown that the process of transcription in genes under selection facilitates the accommodation of mutations that confer escape from non-dividing conditions (Kim et al., 2006; Wright, 2004).

- At sites of stalled transcription, which occurs in the presence of pre-mutagenic DNA lesions, error-prone DNA polymerases may be recruited to the damaged site as a repair mechanism. This specific interaction between transcription and DNA repair is likely to introduce mutations that confer escape from non-growing conditions at transcribed loci.

Research Methods:
- Strategy and Aim: 1) To determine if gyh (polY) and mfi interact either within the same pathway, or in an additive fashion, to influence stationary phase mutagenesis, and 2) To examine the effects of single and double mutants on the stationary phase mutagenesis assay. To construct the double mutant, we introduced a disrupted mfi allele into the B. subtilis strain containing a defective gyh allele.

Double Mutant Strain Construction:
- A 600 bp fragment was PCR amplified out of the wild type strain using primers with EcoR1/MluI overhangs. This segment was then deleted by PCR amplification and ligated into pMUTIN4 (see Fig. 3).

- The resulting plasmid was introduced into E. coli by transformation and resulting isolates were selected on 2% YET agar containing ampicillin (100 mg/mL).

- Constructs were verified with the use of plasmid isolation and restriction analysis. A fragment can be seen at approx. 500 bp (see Fig. 4).

- The plasmid pMUTIN4: mfi was then transformed into YB555: yghTmE strain. Transformants were selected on TEAB agar containing tetracycline (5 mg/mL) and erythromycin (0.3 mg/mL).

- In order to verify possible transformants, primers were designed that anneal to the bacterial chromosome and the defective mfi gene. The PCR products were then run through a gel electrophoresis and bands were visible for two transformants in lanes 3 and 4 (see Fig. 5).

Conclusions & Future Directions:
- The pMUTIN4: mfiTmE plasmid was properly transformed into E. coli.
- The double mutant was constructed by transforming pMUTIN4: mfiTmE into YB555: yghTmE.
- This double mutant strain will then be used in our previously described stationary phase mutagenesis assay, along with the isogenic wildtype strain (YB555), the strain with the disrupted ygh allele, and the strain with the defective mfi allele.

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