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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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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), Yqh (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 Yqh ‘. 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:**

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 in *B. subtilis*, stationary-phase mutants are prone to mutations that confer escape from non-growing conditions. Accordingly, strains lacking transcription factors show a statistically low number of mutations that confer escape from a variety of stress conditions. Moreover, stationary-phase mutants display increased levels of certain stationary-phase genes. It is possible to speculate that when cells are in a stationary phase, perhaps due to pre-mutagenic lesions in the template DNA strand, error-prone DNA polymerases could act to repair these lesions and increase the number of beneficial mutations. This relationship between transcription and DNA repair is likely to play a role in the selection of mutations at a higher frequency than in dividing cells. This study may help in gaining insights into the mechanisms at play in both cell divisions and stationary phases, which may have implications for the development of new drug therapies.

**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 hypervariable subpopulation of cells. These cells limit mutation rate in time and space (Gallardo, 2007).
- In *B. subtilis*, the rpoD gene encodes an error-prone DNA polymerase and is encoded as 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 (Gong et al., 2001; Ross et al., 2006).

**Hypothesis:**

- Previous research has shown that the process of transcription in genes under selection facilitates the accommodation of mutations that confer escape from non-growing conditions (Verma 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 a higher frequency than in dividing cells.

**Research Methods:**

**Strategy and Aim:**
1. To determine if rpoD (polV) and nfo interact either within the same pathway, or in an additive fashion, to influence stationary phase mutagenesis.
2. To compare the effects of stationary phase mutagenesis in wild-type and rpoD knockout strains.

**Double Mutant Strain Construction:**
- A 500-bp fragment was PCR amplified out of the wild-type strain using primers with EcoRI and HindIII overhangs. This segment was then digested and ligated into pMUTIN4 (see Fig. 3).

**Conclusions & Future Directions:**

- The pMUTIN4 plasmid was properly transformed into *E. coli*.
- The double mutant was constructed by transforming pMUTIN4:nfoΔ mutant into *B. subtilis* strain containing a defective nfo allele.

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**References:**