Abstract

*Bacillus subtilis* is a gram positive soil bacteria that is ubiquitous in soil. This organism is used in place of the commonly researched *E. coli*. We are studying DNA mutation during stationary phase (nongrowing) conditions. There is a growing body of scientific research that is suggesting that mutation can occur during transcription of RNA. RNA transcription is used to create proteins that perform the function encoded by DNA. It is an assumption that DNA mutations occur during DNA replication. We have created a temperature sensitive mutation that occurs 90 minutes after the cessation of growth of the strain. We are studying DNA mutation during stationary phase in *B. subtilis* and are using a stationary phase assay to measure the acquisition of mutations. We have shown that stationary phase mutagenesis in *B. subtilis* is independent of genome replication.

Methods

- **Strains**
  - YB955 contains three point mutations that confer auxotrophy in *B. subtilis* (Sung and Yasbin, 2002):
    - metB5
    - recA952 (these are non-sense mutations)
    - leuC427 (this is a missense mutation)
  - KM109 contains the YB955 mutations and a temperature sensitive mutation in *dnaA*, which prevents genome replication at 45°C, but permits replication at 30°C (the strain with the *dnaA* mutation was kindly provided by Jude Wang) (Figure 1)
- **Stationary phase assay**
  - We measure the acquisition of mutations by quantifying the number of cells that have converted from Leu to Leu:
    1. Cells are grown to stationary phase – 90 minutes after the cessation of growth (T90).
    2. Cells are washed and resuspended in SMS salts (contain no nutrients)
    3. Cells are plated on media lacking methionine and leucine (YB955 and KM109 require this amino acids for growth) and incubated at 45°C
    4. At different time intervals (0, 2, 4, 6, 8, and 10 days of incubation) cells are supplied with methionine and incubated at 30°C (this conditions allow growth of Leu+ mutants in have similar numbers of mutants and that the number of mutants increases over time).

Viability assays

To determine whether the strains were differentially affected in viability we collect cells from the plates incubated under stress and at 45°C quantify them by plating them on media containing all required factors for growth and at 30°C. This procedure has been previously described (Pybus et al, 2010).

Results

Figure 2. Stationary phase mutagenesis in *B. subtilis*. A) Accumulation of Leu+ mutations in YB955 and KM109. B) Viability of non-revertant cells, similar rates and conditions with the same mutation rate. Survivals were taken and both maintained the same number of cells living on the plates.

Future Directions

- Extend our mutagenesis analysis to other genetic markers
- Determine the type of mutations generated in the absence of genome replication
- Determine whether DNA repair becomes mutagenic in stationary phase cells.

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