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The Role of recN in stationary phase mutagenesis in bacillus subtilis

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Here, we examine mutagenic programs that are independent of growth, such aspects of the evolutionary process are novel and have been implicated in the formation of cancers in animal cells and the acquisition of antibiotic resistance in animal pathogens. Adaptive or stationary phase mutagenesis is a genetic program to in increase diversity in cells under conditions of stress whereby cells escape non-dividing conditions. Previous research has shown that recombination functions are required to generate mutations that promote growth in *Escherichia coli* cells starved for carbon. This project tests the hypothesis that recombination functions are required for the generation of mutations that promote growth in response to amino acid starvation stresses in *Bacillus subtilis* cells. In *B. subtilis* cells, *recN*, in addition to *recA*, mediates recombination events and may influence the formation of adaptive mutations. A $RecN^-$ strain will be generated by standard molecular techniques and compared to a $RecN^+$ strain for its ability to accumulate mutations that affect amino acid biosynthesis. We speculate that *recN* does not affect stationary phase mutagenesis in *B. subtilis* and discussed other novel mechanisms mediating the generation of mutations in non-dividing cells.

The Role of *recN* in Stationary Phase Mutagenesis in *Bacillus subtilis*

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Abstract

Here mutagenic programs that are independent of growth were examined. Such aspects of the evolutionary process are novel and have been implicated in the formation of cancers in animal cells and the acquisition of antibiotic resistance in animal pathogens. Adaptive or stationary phase mutagenesis is a genetic program to in increase diversity in cells under conditions of stress whereby cells escape non-dividing conditions. Previous research has shown that recombination functions are required to generate mutations that promote growth in *Escherichia coli* cells starved for carbon. This project tests the hypothesis that recombination functions are required for the generation of mutations that promote growth in response to amino acid starvation stresses in *Bacillus subtilis* cells. In *B. subtilis* cells, *recN*, in addition to *recA*, mediates legitimate and illegitimate recombination events and may influence the formation of adaptive mutations. A RecN$^-$ strain was generated by standard molecular techniques and compared to a RecN$^+$ strain for its ability to accumulate mutations that affect amino acid biosynthesis. We report that *recN* affects stationary phase mutagenesis in *B. subtilis* and discussed other novel mechanisms mediating the generation of mutations in non-dividing cells.

Introduction

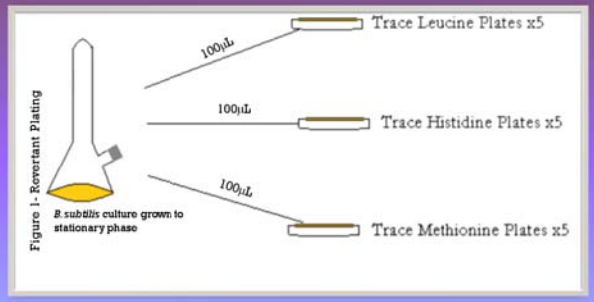
Surprisingly, increasing evidence (dating back to the 1950s) has demonstrated that mutations can arise in non-growing cells as well as in those cells actively involved in the growth process. Furthermore, the mutations that accumulate in the non-growing cells appear to play an important role in the evolutionary process in general. This adaptive or stationary phase mutagenesis is a phenomenon that occurs in non-dividing cells whereby cells mutate in response to sustained stresses on their environment. While it has been proposed that homologous recombination is a process that contributes to genetic diversity, RecA (the protein central to homologous recombination in bacteria) is not an essential component of the stationary phase mutagenesis process in *B. subtilis*. The RecN protein, which is also actively involved in the homologous recombination system has been shown to be involved in processes that both require and that are independent of the RecA protein. Using the bacterium *Bacillus subtilis*, further dissection of the potential role of homologous recombination on stationary phase mutagenesis will be obtained. Using a chloramphenicol cassette, the *recN* gene was inactivated and the effect of this inactivation on stationary phase mutagenesis was determined.

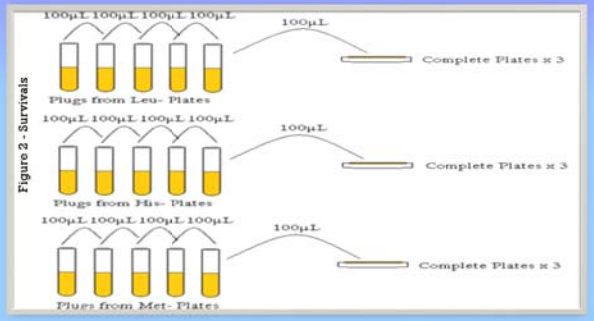
Hypothesis

It is hypothesized that RecN does not contribute to the accumulation of random mutations generated during stationary phase in *Bacillus subtilis*.

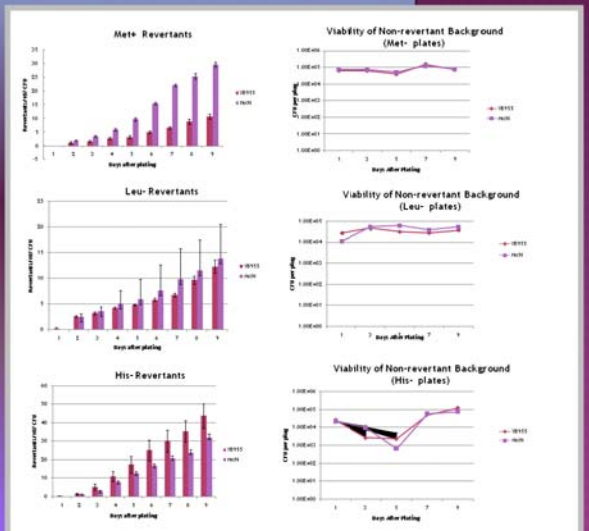
Methods

- Genomic DNA was extracted from *Bacillus subtilis* strain BG281 *(recN::cat)*
- An isogenic *recN* knockout was prepared by transforming the parental strain YB955 with the DNA from BG281.
- Chloramphenicol (cm^R) cassettes within *recN* gene produced a fragment that is 2.2 kb larger than wild type *recN* and also rendered the strain resistant to chloramphenicol.
- Colonies were then isolated on TBAB medium containing chloramphenicol (5µg/m)
- Knockout was verified by PCR and gel electrophoresis
- Sample cultures of wild type and mutant were grown to stationary phase
- The cultures were then plated on minimal media containing trace Histidine, Methionine, and Leucine
- Number of revertants were then scored daily for 9 days while the survival of background cells were monitored every other day





Results



(left): Graphs show the accumulation of revertants on minimal media over a period of nine days.
(right): Graphs show viability of the non-revertant background.

Conclusions/Future Directions

The preliminary data is indicative that *recN* is involved in the stationary phase mutagenesis in *B. subtilis*. However, the influence of this gene is dependent on the genetic event required for cells to escape non-growing conditions in *B. subtilis*. Further analysis is required to elucidate how *recN* influences stationary phase mutagenesis.

These experiments are being repeated and a fluctuation test will also be conducted to determine if *recN* plays an active role in exponential growth.

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