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The Effect of CodY on stationary phase mutagenesis in *Bacillus subtilis*

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Using CodY, a global transcriptional regulator, to modulate transcription and accumulation in genes under selection in *B. subtilis*.

We examine the notion that cells in conditions of stress accumulate mutation in genes under selection via transcription processes. CodY is a global transcriptional regulator in many Gram positives, including soil and pathogenic microbes. In conditions of exponential growth and when branch chain amino acids and GTP are in abundance CodY acts as a transcriptional repressor of many metabolic operons. This transcriptional repression saves the cell energy and allows efficient use of resources. In conditions of starvation, CodY relieves repression of genes involved in acquisition of nutrients and degradation of carbon sources (genes under selection). Here, we compare the accumulation of mutations in genes under selection in wild type and CodY.



The Effect of CodY on Stationary Phase Mutagenesis in *Bacillus subtilis*



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Background

Abstract:

Here we examine the notion that cells in conditions of stress accumulate mutations in genes under selection via transcription processes. CodY is a global transcriptional regulator in many Gram positives, including soil and pathogenic microbes. In conditions of exponential growth and when branch chain amino acids and GTP are in abundance CodY acts as a transcriptional repressor of many metabolic operons. This transcriptional repression saves the cell energy and allows efficient use of resources. In conditions of starvation, CodY relieves repression of genes involved in acquisition of nutrients and degradation of carbon sources (genes under selection). Here, we compare the accumulation of mutations in genes under selection in wild type and CodY.

Introduction:

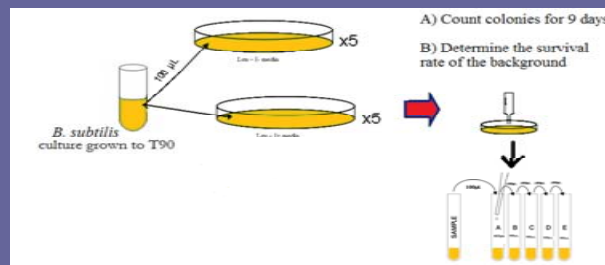
- The study of mutagenic processes provides a more complete view of evolution and insights into molecular mechanisms implicated in the formation of cancers
- Adaptive or stationary-phase mutagenesis occurs in cells under non-growing conditions or when the cells are subjected to non-lethal selective pressure
- This phenomenon has been extensively studied in *Escherichia coli*
- In *E. coli*, the generation of adaptive mutations are dependent on recombination functions, the SOS and stress responses
- In *B. subtilis*, it has been proposed that a physiologically stressed bacterial population differentiates a hypermutable subpopulation and that these hypermutable cells generate mutations randomly (Sung and Yasbin, 2002)
- If one or more of the mutations help the cell survive or grow under stress, then the organism will appear to have "adapted" to its environment
- Other reports also suggest that adaptive mutations in *B. subtilis* are mediated by transcription processes (Robleto et al, 2007)
- Here we examine the concept of transcription-associated mutagenesis by genetically manipulating transcription of a gene under selection and measuring the accumulation of mutations in conditions of repression, derepression, and starvation

Hypothesis:

Inactivating CodY, a global transcriptional regulator, increases transcription of the *ilv-leu* operon, and therefore will result in an increase in stationary phase mutagenesis at the *leuC* allele.

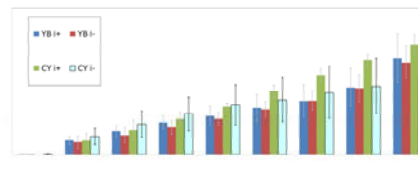
Methods

Strains: *B. subtilis* YB955 and CodY⁻. YB955 has a point mutation in the *leuC* gene, *leuC427*. It consists of a missense mutation (GGA→AGA at position 427). CodY⁻ contains the same *leuC427* mutation as YB955 and a genetic inactivation of CodY, a global transcriptional regulator, that acts as a transcriptional repressor of many metabolic operons, including the one containing *leuC*, in the presence of GTP and amino acids. In conditions of abundance of isoleucine and GTP cells containing CodY repress transcription of *leuC* (Shivers and Sonenshein, 2005).

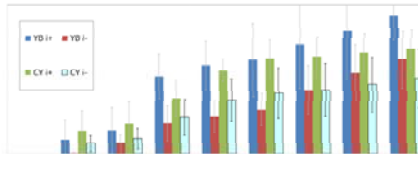


- YB955 and CodY mutant are grown in PB medium at 37 °C to ninety minutes passed the transition to stationary phase.
- The cells were harvested by centrifugation and then resuspended in Spizizen salts.
- Washed cells are plated on minimal media with and w/out isoleucine (I⁻ and I⁺).
- The plates were incubated at 37°C and examined for revertants for nine days.
- Viability of the non-revertant background is assessed during the experiment by taking plugs from the minimal media, performing a serial dilution and plating on complete media supplemented with all amino acids.

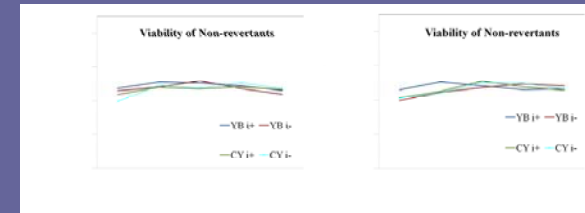
Results



Graph 1 shows that the presence of isoleucine in the environment has no effect on accumulation of Leu⁺ revertants. Also, the CodY⁻ strain has a slightly higher accumulation of Leu⁺ revertants than YB955.



Graph 2 shows adaptive mutagenesis on plates containing extra histidine (the CodY⁻ mutation results in increased degradation of amino acids). YB955 with isoleucine showed a slight increase in the accumulation of Leu⁺ revertants.



Graphs 3 and 4 show that the viability of the cells plated was not affected by the different minimal media.

Conclusions

- Inactivating CodY had a slight effect on stationary phase mutagenesis in conditions of excess of histidine.
- CodY represses transcription in the presence of amino acids.
- Cells in stationary phase are depleted of CodY corepressors, amino acids and GTP.
- Hence, the presence or absence of CodY may not alter transcription in stress conditions.
- Studies in mRNA expression will determine whether transcription of the *leuC* gene is altered by CodY in stationary phase.

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