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Inactivation of *spo0A* gene increases Stationary Phase Mutagenesis in *Bacillus subtilis*

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Abstract

Stationary phase mutagenesis occurs when a population of cells acquires mutations conferring escape from non-growing or stress conditions. This type of mutations is observed in nutritionally starved cells. Because the mutations occur after the onset of stress and in cells that are in non-replicative conditions, elucidating the underlying mechanisms contributes novel views to the process of evolution and apply to the formation of cancer in human cells and antibiotic resistance in microbial pathogens. In *B. subtilis*, entry into stationary phase activates the development of differentiated cell subpopulations that confer competence, cannibalism, antibiotic production, biofilm formation and sporulation. Here we investigate whether the development of endospore formation influences the ability of cells to generate stationary-phase mutations. One key factor in the development of sporulation is the Spo0A transcription factor. We examined the accumulation of mutations in two chromosomal markers in cells that were either Spo0A proficient and Spo0A deficient. Cells that are deficient in Spo0A were significantly increased in accumulation of mutations that confer leucine or methionine prototrophy compared to cells proficient in Spo0A. These results then suggest that activation of the program for the development of endospores prevents the formation of mutations in stationary-phase cells.

Background

- Stationary phase mutagenesis, also known as stress-induced mutagenesis, increases genetic diversity in cells under stressful or non-dividing conditions (first observed in the 1950s by Francis J. Ryan).
- In 1990 this concept was reintroduced and studied in conditions where a *lac*$^-$ *E. coli* strain was starved for a carbon source and observed for its accumulation of lac$^+$ mutations in stress conditions. It was observed that cells accumulated more mutations in conditions of stress than in growth conditions .
- Studies have shown that the *E. coli lacI* system has two mutational pathways, the adaptive point mutation and the adaptive amplification pathway (Rosenberg and Hastings, 2004).
- In *Bacillus subtilis*, studies have shown that the Mfd protein influences the generation of stationary phase mutations, whereas in *E. coli* Mfd does not seem to affect the generation of adaptive mutations.
- Despite the different mechanisms between *B. subtilis* and *E. coli* there are some similarities; error-prone DNA replication influences stress-induced mutations in both systems (Robleto et al. 2007).
- Sporulation by *B. subtilis* is a developmental process that is responsible for the conversion of a growing cell into a dormant cell type known as an endospore. This developmental program gets activated during conditions of stress and results in the production of a cell subpopulation.
- Entry into stationary phase in *B. subtilis* is activated by the Spo0H sigma factor and results in the formation of different cell subpopulations; one of these subpopulations consists of those cells that form spores.
- The master regulator for entry into sporulation in *Bacillus subtilis* is the DNA-binding protein Spo0A, which has been found to influence the expression of over 500 genes during the early stages of development (Molle et al. 2003).
- Here, we investigate the effect of Spo0A on stationary phase mutations that confer methionine or leucine biosynthesis.

Hypothesis

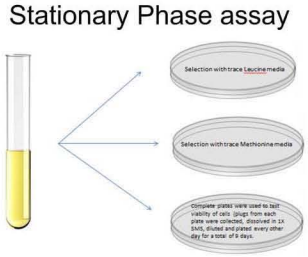
From a germline perspective, the formation of endospores requires high fidelity DNA replication to ensure genome integrity. Thus, inactivating the sporulation gene spo0A increases the number of mutations in stationary phase mutagenesis in *Bacillus subtilis*.

Methods

Strains used:

- **AAP101 (MF476→YB955)**
- **YB955 (*hisC952 metB5 leuC 427 xin spβ*^{SENS})**

Strain AAP101 was constructed by isolating DNA from strain MF476 and transforming strain YB955 as previously described in Boylan et al. paper in 1972. Transformants were selected on plates containing erythromycin at concentration of 1µg/mL)



References

1. Boylan, R. J., N. H. Mendelson, D. Brooks, and F. E. Young. 1972. Regulation of the bacterial cell wall: analysis of a mutant of Bacillus subtilis defective in biosynthesis of teichoic acid. J. Bacteriol. 110:281–290.
2. Molle, V., Fujita, M., Jensen, S.T., Eichenberger, P., Gonzalez-Pastor, J.E., Liu, J.S., Losick, R. 2003. The Spo0A regulon of *Bacillus subtilis*. *Molecular Microbiology*. 50: 1683-1701.
3. Robleto E.A., Yasbin, R., Ross, C., Pedraza-Reyes, M. 2007. Stationary Phase Mutagenesis in *B. subtilis*: A Paradigm to Study Genetic Diversity Programs in Cells Under Stress. *Critical Reviews in Biochemistry and Molecular Biology*. 42:327-339.
4. Rosenberg, S.M, Hastings, P.J. 2004. Adaptive Point Mutation and Adaptive Amplification Pathways in the *Escherichia coli* Lac system: Stress Responses Producing Genetic Change. *Journal of Bacteriology*. 186:4838-4843.

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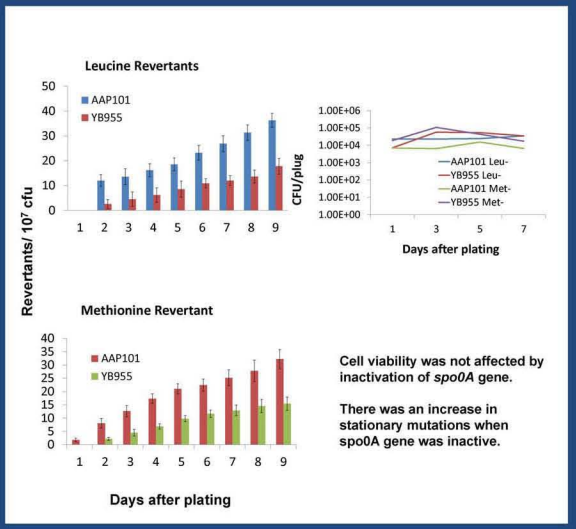
Strain MF474 were a kind gift from The laboratory of Dr. Masaya Fujita at the University of Houston.

Results



Cell viability was not affected by inactivation of *spo0A* gene.

There was an increase in stationary mutations when spo0A gene was inactive.

Conclusions

- Inactivation of the sporulation gene *spo0A* increases stationary phase mutagenesis in *Bacillus subtilis*.
- Activation of the sporulation program inhibits development of stationary phase mutations.

Future Directions

- Examine revertants at the Histidine allele.
- Examine the effect of single inactivation of Spo0H and in combination with Spo0A on stationary phase mutagenesis.