Stationary phase mutagenesis in *Bacillus subtilis* is independent of genome replication

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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 will not allow the DNA helicase to bind to DNA to open it up for replication. This should prevent the beginning of DNA duplication there by implying that the mutations that occur are a result of a different mechanism.

Background

•Stationary phase mutagenesis is the process by which cells under non-growing conditions accumulate mutations in genes under selection
•This type of cellular programs are associated with the formation of neoplasia in animal cells and with acquisition of antibiotic resistance and evasion of immune responses in in microbial pathogens (Galhardo, 2007)
•This process have been extensively studied in *Escherichia coli* and indicate that stress-induced, or stationary phase mutations are generated during the processing of intermediates formed during genome replication (Galhardo, 2007; Hastings, 2007)
•In *Bacillus subtilis*, however, several factors required for the processing of replication intermediates are not required and suggest that stationary phase mutations are produced by a different mechanism (Robleto et al, 2007)
•Here we examine stationary phase mutagenesis in the absence of genome replication and showed that *B. subtilis* produces mutations in non-growing cells by mechanisms that do not require genomic replication.

Hypothesis

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, *hisC952* (these are non-sense mutations)
leuC427 (this is a mis-sense mutation)
-KM109 contains the YB955 mutations and a temperature sensitive mutation in *dnaB*, which prevents genome replication at 45 C, but permits replication at 30C (the strain with the *dnaB* mutation was kindly provided by Jade 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 (T_{90})
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 procedures have been previously described (Pybus et al, 2010).

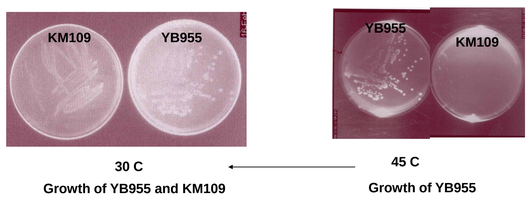


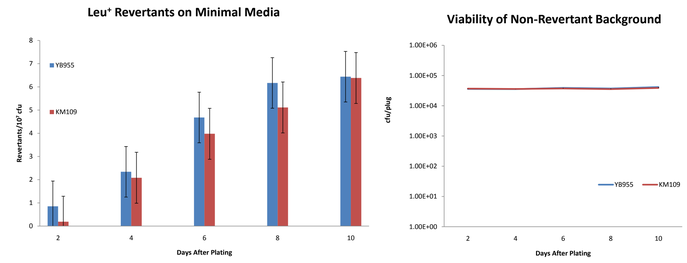
Figure 1. Growth properties of the strains used in this study.

References

• Gallhardo, R., Hastings, P.J., Rosenberg, S. 2007. Mutation as a Stress Response and the Regulation of Evolvability. *Critical Reviews in Biochemistry and Molecular Billogy 42:399-435*
2. Robleto,E.A., Yasbin, Y., Ross,C., and 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
3. Sung,M.,Yeamans, G., Ross, C.A., and Yasbin, R.E. Roles of YqjH and YqjW homologues of *E. coli* Umu/Din B or Y superfamily of DNA polymerases, in stationary-phase mutagenesis and UV-induced mutagenesis of *Bacillus subtilis* . *J Bacteriol.* 185: 2153-2160.
4. Pybus, C., Pedraza-Reyes, M., Ross, C.A., Martin, H., Ona, K., Yasbin, R. E. &Robleto, E.2010. Transcription-associated mutation in *Bacillus subtilis* cells under stress. *J Bacteriol.* 192:3321-3328
5. Hastings, P.J. 2007. Adaptive amplification. *Crit Rev Biochem Mol Biol.* 42, 271-83

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.



Summary : 1) both strains accumulated mutations similarly which suggest that genome replication is not required for the formation of mutations in resting cells. 2) Viability of non-revertant cells is not affected and does not influence the abilty to accumulate mutations similar rates and conditions with the same mutation rate. Survivals were taken and both maintained the same number of cells living on the plates.

Conclusions

Stationary phase mutagenesis in *B. subtilis* occurs in the absence of genome replication.

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.

Acknowledgements

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