Aug 3rd, 9:00 AM - 12:00 PM

Antibiotic resistance in Bacillus subtilis as affected by transcriptional derepression and the stringent response

Brandon Eisinger
University of Nevada, Las Vegas

Ronald E. Yasbin
University of Nevada, Las Vegas

Eduardo A. Robleto
University of Nevada, Las Vegas

Antibiotic Resistance in *Bacillus subtilis* as Affected by Transcriptional Derepression and the Stringent Response

Brandon Eisinger, Ronald E. Yasbin, and Eduardo A. Robleto
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV

Abstract:

Bacterial cells under conditions of starvation or prolonged non-lethal selective pressures accumulate mutations in highly transcribed genes. This process is part of cellular programs to increase genetic diversity in conditions of stress, also known as stationary phase or stress-induced mutagenesis. This experiment investigated mutation frequencies for antibiotic resistance as affected by the stringent response. The stringent response is a global cellular process that initiates at the cessation of growth and mediates changes in gene expression that repress synthesis of ribosome components. We used *Bacillus subtilis* strains that differ in RelA proficiency. The *relA* gene controls the synthesis of (p)ppGpp, the signaling molecule which mediates the stringent response. Since genes involved in protein synthesis are repressed during the stringent response, we hypothesize that relaxed mutants express a higher accumulation of mutations that confer resistance to tetracycline than cells that become stringent. Resistance to tetracycline may be acquired by altering components of the small subunit of bacterial ribosomes. Utilizing an overlay procedure and increasing times of incubation under nutritional stress, stationary cells were prompted for resistance to tetracycline. Our results showed that *relA*$^-$ cells expressed a higher accumulation of Tc^r mutations than the one observed in wild type cells. These results provide evidence that transcriptional derepression in cells under non-lethal stress mediates mutagenic events. Implications in antibiotic resistance are further discussed.

Background:

- *B. subtilis* cells under conditions of starvation or prolonged nonlethal selective pressures (e.g., starvation for amino acids or carbon source, exposure to high temperatures, extreme pH, increased DNA damage, etc) accumulate stress-induced mutations in highly transcribed genes.

- Under starvation conditions, cells will initiate the stringent response. This causes the cell to divert resources away from growth and division, resulting in repression of genes involved in the synthesis of translational machinery such as rRNA and tRNA. This is accomplished by inhibiting RNA polymerase to transcribe rRNA/tRNA promoters. Concomitant with this transcriptional repression, there is a an increase in transcription of biosynthetic genes such as amino acid synthesis as a response to nutrient limitation.

- RelA$^-$deficient strains show no stringent response because they lack synthesis of (p)ppGpp, a key intermediate for the inset of the stringent response. (reviewed in Eymann, et. al, 2002).

Hypothesis:

Since genes involved in protein synthesis are repressed during the stringent response, we hypothesize that relaxed mutants accumulate more mutations that confer resistance to tetracycline than cells which are stringent (Figure 1). Utilizing an overlay procedure and increasing times of incubation under nutritional stress, stationary cells were tested for resistance to tetracycline.

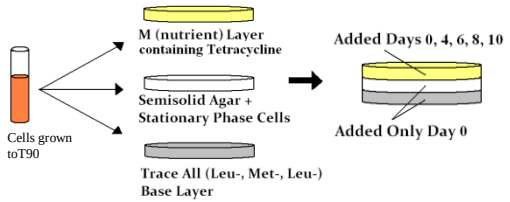
Wild Type **(YB955)**
↓
Amino Acid or Carbon Starvation
↓
Global Stringent Response
↓
(p)ppGpp Synthesis Increases
↓
rRNA and tRNA Synthesis Decreases
↓
Amino Acid Biosynthesis Increases

relA$^-$ cells
↓
Amino Acid or Carbon Starvation
↓
Relaxed (non-stringent) State
↓
No Increase of (p)ppGpp
↓
Derepression of rRNA and tRNA synthesis
↓
Increased Mutation Rate

Methods:

Bacterial strain YB955 used in this study is auxotrophic for Met, Leu, and His. The YB955 *relA*::erm mutant is isogenic to the wild type strain but contains an erythromycin (erm) cassette insertion resulting in a null mutation of the RelA and loss of (p)ppGpp synthetase activity. Both strains were grown aerobically at 37ºC using PAB broth until the cells reached stationary phase (measured by optical density). Minimum inhibitory concentration of tetracycline was determined to be 10µg/ml up to a 72hr incubation period. Tetracycline-resistant colonies were scored at various days utilizing an overlay procedure.

Fig. 2 Assay for Stationary Phase Mutagenesis in *B. subtilis*



Layer 1 (base) contained trace Met$^-$Leu$^-$His$^-$. Layer 2 contained semisolid agar plus test strain. Layer 3 contained M (nutrient) media and was added to cells starved to different time intervals. Viability of the cells throughout the experiment was measured by taking plugs from starved cells on select days and plating them on complete media.

Results:

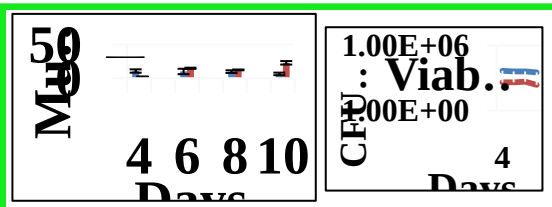


Fig. 3. (a) Accumulation of Tc^r mutations in YB955 and in YB955 relA::erm over a 10 day period on overlay media. Error bars represent standard error of the mean. **(b)** Viability of non-mutant background using plug method on specified days.

Conclusions:

•The wild type YB955 strain consistently grows 10-fold higher than the YB955 *relA*::erm mutant strain, this is consistent with the idea that stringent cells are better adapted to starvation.

• Over time, YB955 accumulates less Tc^r mutations than YB955 *relA*::erm under conditions of stress. This result is consistent with the idea that the highly transcribed regions in cells under conditions of stress are prone to mutagenic processes. This also implies that antibiotics that target processes other than protein synthesis are more likely to occur in cells placed under stress.

Future Directions

• Sequence the Tc^r mutant colonies to determine where the gene mutation occurred

• Determine the role of other ppGpp(p) synthetases and their role in antibiotic resistance.

Acknowledgements:

I would like to thank Holly Martin for her guidance on data input and overall assistance on this project This project is supported by grants MCB0843606, GM072554, and 2 P20 RR016463 Nevada INBRE.

References:

• Inaoka, T., & Ochi, K. (2002). RelA Protein Is Involved in Induction of Genetic Competence in Certain *Bacillus subtilis* Strains by Moderating the Level of Intracellular GTP. *J Bacteriol* 184:3923-3930.

• Robleto, E.A., Yasbin, R., 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.

• Gentry, D., Li, T., Rosenberg, M., and McDevitt, D. (2000). The *rel* Gene is Essential for In Vitro Growth of Staphylococcus aureus. *J Bacteriol* 182:4995-4997.

• Lopez, J. M., Dromerick, A., and Freese, E. (1981) Response of Guanosine 5'-Triphosphate Concentration to Nutritional Changes and Its Significance for *Bacillus subtilis* Sporulation. *J Bacteriol* 146:605-613.

• Eymann, C., Homuth, G., Scharf, C., and Hecker, M. (2002) *Bacillus subtilis* Functional Genomics: Global Characterization of the Stringent Response by Proteome and Transcriptome Analysis. *J Bacteriol* 184:2500-2520.

• Natori, Y., Tagami, K., Murakami, K., Yoshida, S., Tanigawa, O., Moh, Y., Masuda, K., Wada, T., Suzuki, S., Nanamiya, H., Tozawa, Y., and Kawamura, F. (2009). Transcription Activity of Individual *rrn* Operons in *Bacillus subtilis* Mutants Deficient in (p)ppGpp Synthetase Genes, *relA*, *yjbM*, and *ywaC*. *J Bacteriol* 191:4555-4561.

• Metzger, S., Dror, I.B., Aizenman, E., Schreiber, G., Toone, M., Friesen, J.D., Cashel, M., and Glaser, G. (1988). The Nucleotide Sequence and Characterization of the *relA* Gene of *Escherichia coli*. *Journal of Biological Chemistry* 263:15699-15704.