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

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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 mutation. 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 the B. 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 hypothesized that relaxed mutants accumulate 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 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.

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.

Methods:

Bacterial strain YB955 used in this study is isogenic 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 37°C using PAB broth until the cells reached stationary phase (measured by optical density). Minimum inhibitory concentration of tetracycline was determined to be 0.1µg/ml up to a 72h incubation period. Tetracycline-resistant colonies were scored at various days utilizing an overlay procedure.

Results:

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 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 mutant colonies to determine where the gene mutation occurred

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

References:

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Fig. 3. (a) Accumulation of Tc 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-starved background using plug method on specified days.

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