Fatty Acid Starvation and the Stringent Response in the Gram-positive Model Bacterium *Bacillus subtilis*

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Introduction: In the natural environment, bacteria are constantly undergoing periods of stress to which they must adapt. In Gram-negative bacteria, the small molecule alarmone guanosine-5-diphosphate-3-diphosphate (ppGpp) is produced in response to one such stressor, lipid starvation. However no information is available about the response to lipid starvation in Gram-positive bacteria. **Objective:** The main objective of this work was to evaluate the role played by the stringent response during fatty acid starvation in *Bacillus subtilis*. **Material and Methods:** Mutants bearing deletions of genes involved in ppGpp synthesis were used to evaluate the consequences of fatty acid deprivation (induced by the antibiotic Cerulenin). Morphology alterations were observed by fluorescence microscopy using membrane and DNA dyes. Viability was estimated by CFU/mL count. Nucleotide and ppGpp levels were measured by thin-layer chromatography. **Results and Discussion:** Removing ppGpp synthesis capability of *B.subtilis* causes severe viability loss when facing fatty acid starvation (99,9% decrease, compared to wild-type), mainly due to membrane damage and loss of membrane potential. Differently from the fatty acid deprivation response in Gram-negative bacteria, we did not observe increases in ppGpp levels. However, major changes in GTP and ATP levels were observed. Decreasing the GTP/ATP ratio using a specific drug (decoynine) increased viability of the ppGpp null strain. **Conclusion:** Fatty acid deprivation, a situation that normally has bacteriostatic effects, became bactericidal when *B.subtilis* lacked stringent response proteins, due to severe membrane dysregulation. Although we did not detected shifts in ppGpp levels, GTP and ATP levels are greatly altered during fatty acid starvation. This is in agreement with recent indications that the stringent response in Gram-positive bacteria is more dependent on the GTP/ATP ratio than ppGpp, since such nucleotides directly modulate two important factors in maintaining cell viability during stressful conditions: gene transcription and growth rate.

Key words: stringent response, *Bacillus subtilis*, fatty acids, ppGpp, nucleotides, membrane.
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