The bacterial RNA chaperone Hfq is involved in the oxidative stress response of *Listeria monocytogenes*

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Abstract

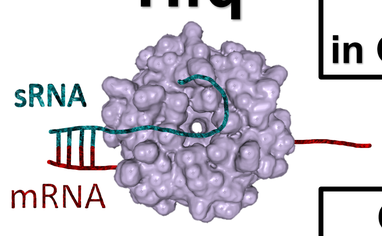
The RNA chaperone Hfq is an important bacterial post-transcriptional regulator. In Gram-negative bacteria its main role is in riboregulation, promoting the interaction between non-coding sRNA and mRNA, a function that was also associated with stress response in *E. coli* and *Salmonella*. However, in Gram-positive bacteria like *Listeria monocytogenes*, Hfq main role remains elusive as this protein seems to be expendable for riboregulation. Here we found that Hfq is essential for the oxidative stress response of *Listeria*, with disruption of the hfq gene resulting in a hypersensitive phenotype to hydrogen peroxide (H_2O_2). Using a sub-inhibitory concentration of H_2O_2, the growth of the wild-type strain was barely affected and recovered immediately upon addition of the oxidative stressor. However, addition of this same concentration to the hfq null-mutant culture resulted in growth inhibition from which the strain could not recover even 12h after stress. H_2O_2 is one of the several reactive oxygen species (ROS) found inside cells. H_2O_2 toxicity arises from the production of $OH^{\cdot}$ radicals that are extremely deleterious to several molecules. As catalase is the major enzyme involved in the decomposition of H_2O_2, we hypothesised that the increased sensitivity to H2O2 of the hfq mutant could result from a less active catalase. In fact, an activity assay demonstrated that catalase is 30% less active in the absence of Hfq when compared to the wild-type. Being a facultative intracellular pathogen, *Listeria* faces oxidation inside phagocytic cells, as the production of ROS is key in their antibacterial activity. The results obtained here connect Hfq regulation and the way *Listeria* copes with oxidative stress, given that adaptation to these circumstances is critical for *Listeria* pathogenesis.

Background

Listeria monocytogenes

- Gram-positive, human pathogen
- Facultative intracellular bacteria
- Causative agent of listeriosis
- Colonizes several cell types, including phagocytic cells

Hfq



RNA Matchmaker in Gram-negative bacteria

Controversial role in Gram-positive bacteria

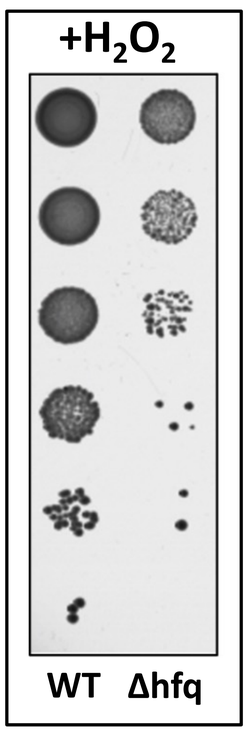
Oxidative stress

- Caused by exposure to high levels of reactive oxygen species (ROS)
- ROS are extremely deleterious to cells
- Phagocytic cells use ROS to kill intracellular bacteria (stress condition)

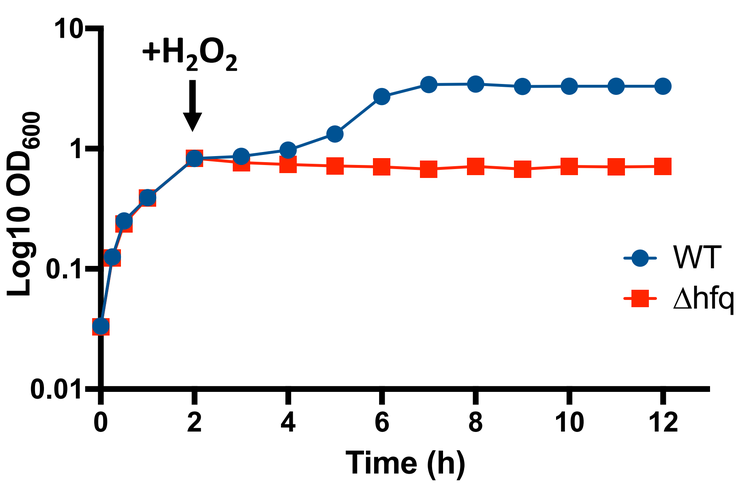
Can Hfq be a stress response protein in *L. monocytogenes*?

Results

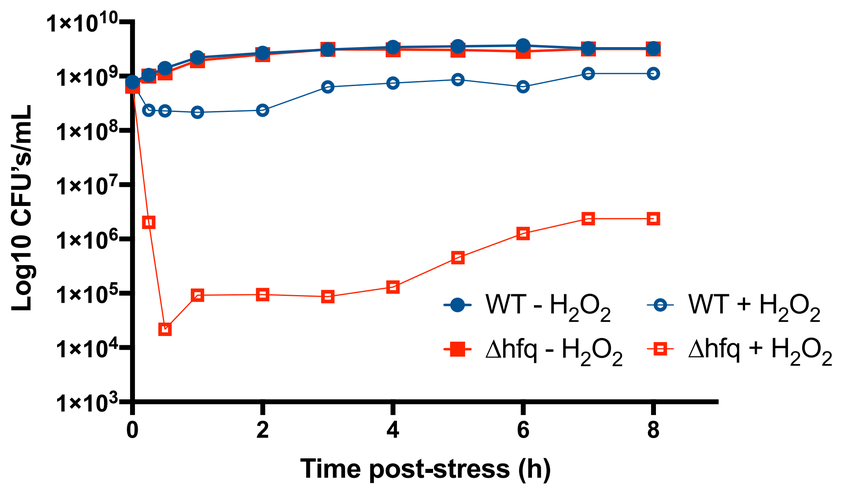
1. Hydrogen peroxide sensitivity

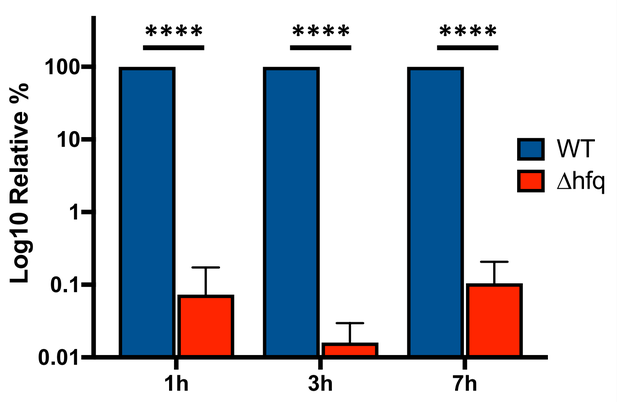


Growth



Viability

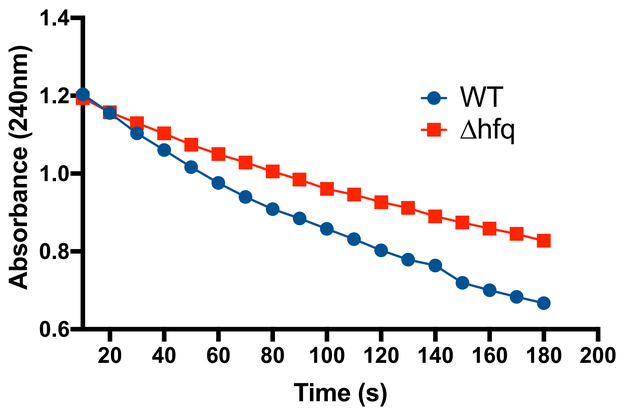


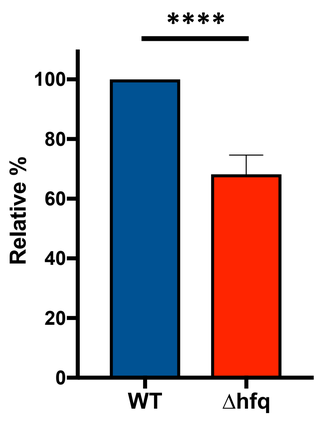


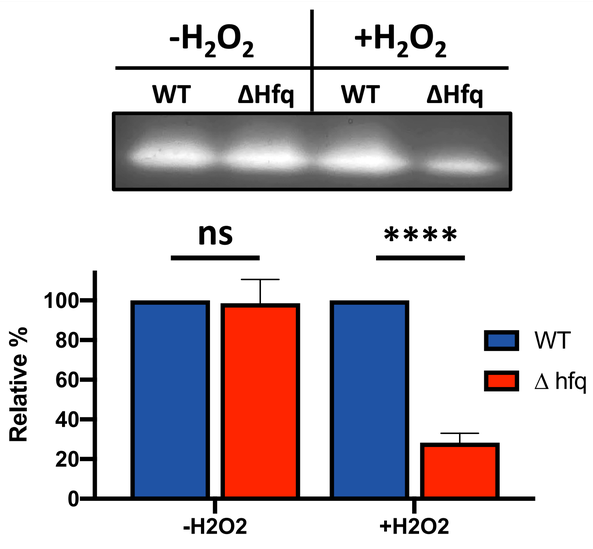
Hfq mutant is more sensitive to hydrogen peroxide

2. Catalase activity

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

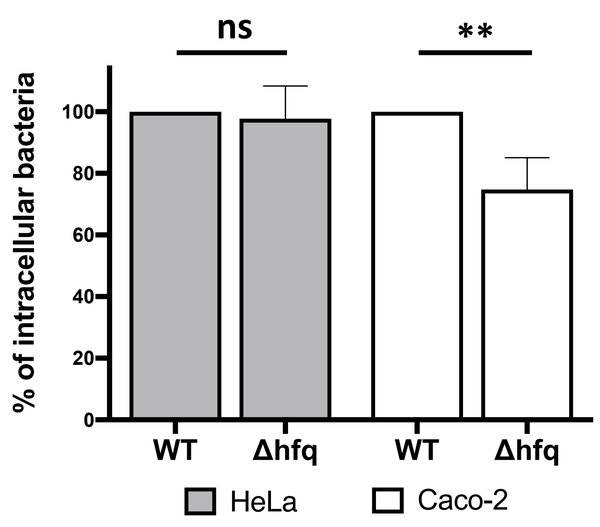






Catalase is ~30% less active in Hfq mutant

3. Virulence



Hfq impacts virulence

Concluding remarks

- **Hfq is a stress response protein in *L. monocytogenes***
- **Hfq is essential in H_2O_2 oxidative stress, and regulates *L. monocytogenes* catalase**

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