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The oxidative stress response of *Francisella tularensis*

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Abstract
Francisella tularensis is capable of infecting numerous cell types, including professional phagocytes. Upon phagocytosis, *F. tularensis* resides within the phagosome before escaping into the cytosol to replicate. Phagocytes constitute a hostile environment rich in ROS, which are employed as a means of killing pathogens. ROS interact with and disrupt the function of vital molecules such as DNA, proteins and bacterial structures. Iron potentiates the danger of ROS through the Fenton reaction where ferrous iron reduces H_2O_2 causing the formation of highly reactive hydroxyl radicals and anions. Low levels of ROS are formed during normal aerobic metabolism and pathogens thus have a need for defense mechanisms to handle the ever present levels of ROS but even more so to combat the onslaught of ROS experienced within a host.

This thesis was focused on the investigation of the iron status and oxidative stress response of *F. tularensis*; thereby identifying key players controlling the bacterial iron content, its adaptation to oxygen-rich environments and defense against ROS.

We identified subspecies-specific differences in iron content, where *F. tularensis* subsp. *tularensis* was found to contain significantly less iron than strains of subsp. *holarctica*. The reduced iron content resulted in an increased tolerance to H_2O_2, despite simultaneously causing a decrease in the activity of catalase - the iron-dependent enzyme responsible for degrading H_2O_2 in *F. tularensis*. This strongly suggests that the restricted iron uptake and storage by subsp. *tularensis* strains is beneficial by rendering the bacteria less susceptible to H_2O_2, thereby evading the toxic effects of the iron driven Fenton reaction. This evasion is likely to be an important part of the higher virulence displayed by subsp. *tularensis* as compared to subsp. *holarctica*.

We further identified that the global regulator, MglA, is important for the adaptation of LVS to oxygen-rich environments. Deletion of *mglA* from LVS resulted in a mutant, Δ*mglA*, with impaired defense to oxidative stress, as manifested by an inability to grow to wild-type levels under aerobic conditions, an accumulation of proteins with oxidative damage, a suppressed expression of iron-uptake related genes, an increased catalase activity, and an increased tolerance to H_2O_2. We therefore conclude that MglA is an important factor for the defense of LVS to oxidative damage under aerobic conditions and speculate that MglA is of greatest importance in oxygen-rich foci.

We also studied the role of OxyR in LVS by creating a Δ*oxyR* mutant as well as a double mutant, Δ*oxyR*/Δ*katG*. The *in vitro* response of these mutants, as well of Δ*katG*, to defined ROS was assessed using H_2O_2, the O_2^- generating agent paraquat, and the $ONOO^-$ generator SIN-1. Δ*oxyR* was more susceptible to all ROS than LVS as was Δ*katG*, with the expection of O_2^-. Strikingly, Δ*oxyR*/Δ*katG* was significantly more susceptible to all ROS tested compared to either single deletion mutant. LVS, Δ*oxyR* and Δ*katG* replicated efficiently in bone marrow-derived macrophages whereas Δ*oxyR*/Δ*katG* showed no replication. In mice, the Δ*oxyR* mutant displayed impaired replication in liver, but intact replication vs. LVS in spleen. Collectively, our results demonstrate an important role of OxyR in the oxidative stress response and virulence of *F. tularensis*, and further reveale overlapping roles of OxyR and catalase in the defense against ROS. The results thus shed new light on the complexity of ROS defense in *F. tularensis*.

Keywords
Francisella tularensis, FupA, MglA, OxyR, ROS, oxidative stress

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