Role of the *bsr* gene in the Intracellular Survival of *Bordetella bronchiseptica*

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**Introduction**

*Bordetella pertussis* and *B. parapertussis* are Gram-negative bacteria that cause whooping cough in humans, while *B. bronchiseptica* (BB) causes bronchitis in mice, dogs, and horses. The wild type strain of BB, RB50, contains a gene (*bsr*) encoding a putative sigma factor that is up-regulated when BB is exposed to blood. To test the role of this gene in pathogen-host interactions, a knock-out mutant called RB50Δbsr was made in our lab. Preliminary results showed that RB50Δbsr survives longer within macrophages (immune cells which engulf and destroy pathogens) than RB50. The mutant also confers sterilizing immunity against further BB and B. pertussis infection in mice, which are excellent models for human infection.

The aim of this study is to determine if there is a difference between how RB50 and RB50Δbsr are internalized by macrophages. We believe there is a delay in lysosome formation in RB50Δbsr-infected macrophages and that RB50Δbsr causes less damage to these immune cells, suggesting that the *bsr* gene can be targeted to produce a better vaccine against Bordetella infections.

**Methods & Materials**

Mouse macrophages in 96-well plates (RPMI media) were challenged with either RB50 or RB50Δbsr bacteria at MOI 100. The cells were then incubated at various time points. For time points greater than 15 minutes, gentamicin (300 μg/ml) was added to the cell media after 1 hour of incubation. The following assays were conducted:

- Confocal microscopy (Fig. 1 & 2) using fluorescent-labeled bacteria to determine co-localization with endosomes or lysosomes
- Phagocytosis assay (Fig. 3) to study the survival of engulfed bacteria over time
- Intracellular assay (Fig. 5) to quantify live bacteria within macrophages over a 24-hour period
- Cytotoxicity assay (Fig. 6) to evaluate macrophage death caused by bacteria
- Electron microscopy (Fig. 7 & 9) to quantify live bacteria within macrophages at specific time points

**Results**

- Figure 1: a) RB50 (green) localizes with lysosome marker LAMP (red) while b) RB50Δbsr does not.
- Figure 2: Less endosomal marker EEA1 (green) surrounds a) RB50 (red) than b) RB50Δbsr (red).
- Figure 3: Bacteria were stained with pHRodo Red, which produces an absorbance signal in acidic environments, such as lysosomes. The absorbance OD value is greater for RB50-challenged macrophages.
- Figure 4: Levels of RB50 and RB50Δbsr are a) stable at 15 minutes, followed by b) an increase in RB50Δbsr at the 4 hour mark. After d) 12 hours, levels of intracellular RB50Δbsr are higher than levels of RB50.
- Figure 5: More RB50Δbsr is recovered from macrophages at all time points. After 12 hours, RB50Δbsr levels begin to rise while RB50 levels drop.
- Figure 6: Less death is observed among RB50Δbsr challenged macrophages than RB50-challenged cells at both time points.
- Figure 7: Individual macrophages (with bacteria in red) at 15 minutes. a) RB50 remains outside the cells, while b) RB50Δbsr is internalized.
- Figure 8: Phagocytosis from 5 to 14 hours of incubation.
- Figure 9: After a) 15 minutes, a greater number of macrophages engulf RB50Δbsr than RB50. This trend continues after b) 4 hours, with some macrophages engulfing more than 10 RB50Δbsr.

**Conclusions**

- More RB50Δbsr than RB50 survives within macrophages overtime.
- RB50 enters into lysosomes readily while RB50Δbsr does not show the same behavior after internalization.
- Infection by RB50Δbsr results in less macrophage death overtime than infection by RB50.

Based on the results of this study, *bsr* plays a vital role in macrophage response to BB infection. Since macrophages are involved in activating several other immune system components, manipulating *bsr* leads to an overall change in the persistence of *Bordetella* infections.

**References**


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