Differences in Pathogenesis for *Salmonella enterica* serovar Typhimurium in the Mouse Versus the Swine Model Identifies Bacterial Gene Products Required for Systemic but not Gastrointestinal Disease

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Abstract

Over the last several decades, the mouse model of typhoid fever has been an extremely productive model to investigate *Salmonella enterica* serovar Typhimurium (S Typhimurium) pathogenesis. The mouse is the paradigm for investigating systemic disease due to infection by *Salmonella*; however, the swine model of gastrointestinal colonization and enteric disease due to *Salmonella* is better suited to address food safety. Although certain *Salmonella* mutants may be attenuated for colonization and disease in both the mouse and swine model, others may only be attenuated in one of the models depending on whether the gene product is required for gastrointestinal or systemic disease. Recent research performed on the swine model in our laboratories with comparison to the literature on the mouse model, illustrates the discrepancy between these two models. The *fepA* *iroN* and *cira* gene products of *S. Typhimurium* are required for iron acquisition by uptake of enterochelin, salmochelin and their breakdown products. In the mouse model, a *S. Typhimurium* mutant with deletions in the *fepA* *iroN* and *cira* genes is attenuated for disease. In contrast, no significant difference in gastrointestinal colonization or fecal shedding was found comparing a *S. Typhimurium* *fepA* *iroN* *cira* mutant to wild-type in the swine model. Furthermore, investigation of a two-component system for sensing the bacterial environment has demonstrated that inactivation of the *qseBC* (*preAB*) genes has a two-day delay in mouse mortality and a competitive defect in the liver and spleen compared to wild-type *S. Typhimurium*. However, in the pig, no significant difference was observed in fecal shedding or gastrointestinal colonization between the *qseBC* double mutant and the wild-type strain. The difference between these two models suggest roles in systemic but not gastrointestinal disease for these *S. Typhimurium* gene products and highlights the importance of utilizing the swine model for the development of *Salmonella* interventions to enhance pork safety.

Introduction

The mouse model of typhoid fever is the paradigm for investigating *Salmonella enterica* pathogenesis in vivo. The combination of in vitro cell culture and the mouse model of typhoid fever has been extremely important in the elucidation of virulence mechanisms utilized by *Salmonella enterica*, especially serovar Typhimurium. A complex repertoire of virulence genes is required for *S. Typhimurium* pathogenicity including gene products encoded in the *Salmonella* pathogenicity islands (SPI) (Coburn et al., 2007). For example, a subset of SPI-1 gene products are required for bacterial invasion of epithelial cells in the gastrointestinal tract, whereas SPI-2 gene products are required for systemic infection and intracellular survival.

Many of the investigations that have utilized the mouse model for *S. Typhimurium* systemic disease have been performed in inbred mouse strains (C57BL/6 and BALB/c) that have increased susceptibility to *Salmonella* due to an inactivation of solute carrier family 11a member 1 (Slc11a1; formerly Nramp-1) (Stecher et al., 2006). Although these inbred mouse strains are not considered natural hosts, there are several advantages to utilizing the murine model; the small rodent size permits minimal effort during animal handling, smaller animal housing facilities, and decreased expense for procurement of animals. In contrast, many larger animals (poultry, swine and cattle) that are natural hosts for *Salmonella* colonization and infection are outbred animals resulting in genetic variability between the individual animals (Grassl and Finlay, 2008). Therefore, significant differences may be seen in these natural hosts for pathogen colonization and disease susceptibility.
Since most of the *Salmonella* pathogenicity studies have been performed in the mouse model, most of the potential interventions to prevent *Salmonella* colonization and disease in food producing animals originate from murine investigations. However, the colonization and pathogenicity of most *Salmonella* serovars is not systemic but limited to the gastrointestinal tract in natural hosts including swine. Additionally, the genetic diversity within the swine population results in variable colonization potential for *Salmonella*. Indeed, we investigated the involvement of specific genetic systems of *S. Typhimurium* for colonization potential in swine and have observed differences between the results that we obtained in swine compared to results published for the mouse model of typhoid fever.

**Material and methods**

**Bacterial strains and media.** The *S. Typhimurium* strain χ4232 and its derivatives were used in this study and all except BBS 192 (*qseBC*) have been previously described (Bearson and Bearson, 2008; Bearson et al., 2008). Strains were grown in Luria-Bertani (LB) broth at 37 °C.

**Swine colonization experiment.** To determine the colonization potential of a *qseBC* double mutant in the swine gastrointestinal tract compared to the *qseC* mutant and wild-type *S. Typhimurium*, 7-week old piglets (n=5 per strain) were intranasally inoculated with the *S. Typhimurium* strains. Fecal shedding of the bacterial strains was monitored for 7 days post-inoculation (dpi) using quantitative and qualitative *Salmonella* culturing methods.

**Quantitative and qualitative *Salmonella* culture analysis.** For quantitative bacteriology, one gram of pig feces was combined with 5 ml PBS, vortexed and 100 μl directly plated to brilliant green agar with sulfadiazine (BGS) containing nalidixic acid (30 μg/ml), ferric ammonium citrate (0.8 g/L) and sodium thiosulfate (6.8 g/L). One hundred microliters of a ten-fold dilution of each fecal sample were also plated, and additional dilutions were performed when necessary. Following 48 hours of incubation at 37°C, black colonies were enumerated and a single colony from each plate was confirmed to be *Salmonella* by serogroup antiserum agglutination.

Qualitative bacteriology of *Salmonella* was performed as follows: 1 gram fecal samples were inoculated in 10 ml of GN-Hajna (GN) broth and tetrathionate (TET) broth for 24 and 48 hours of growth at 37°C, respectively. Following incubation, 100 μl of each culture was transferred to 10 ml Rappaport-Vassiliadis medium (RV) and incubated at 37°C for 18 h. The cultures were streaked on BGS containing nalidixic acid (30 μg/ml), ferric ammonium citrate (0.8 g/L) and sodium thiosulfate (6.8 g/L). Colonies suspicious for *Salmonella* were confirmed by serogroup antiserum agglutination.

**Results and Discussion**

Bacterial two-component systems sense environmental signals and integrate the signals to modulate gene expression or enzyme activity. The classical two-component system features a sensor kinase located in the bacterial inner membrane to detect an environmental signal and a cytoplasmic response regulator to modulate gene expression. For the QseBC (PreAB) two-component system in *S. Typhimurium*, QseC is the membrane sensor kinase and QseB is the cytoplasmic response regulator. In a previous investigation of the QseC two-component system, we demonstrated that the competitive fitness of the *qseC* mutant was decreased compared to wild-type *S. Typhimurium* for colonization of the swine gastrointestinal tract when pigs were co-inoculated with the bacterial strains at a 1:1 ratio (Bearson and Bearson, 2008). To further examine the QseBC (PreAB) two-component system, a *qseBC* double mutant was constructed to compare to the *qseC* and wild-type strains in swine to determine gastrointestinal fitness. Inactivation of the QseC (PreB) sensor kinase significantly decreased fecal shedding of the *qseC* mutant compared to wild-type *S. Typhimurium* at 2, 3, 5 and 7 dpi and the *qseBC* double mutant at 2, 3 and 5 dpi (*P*<0.01) (Figure 1). This data confirms the previous competition data in swine and indicates that QseC is a positive regulator of gastrointestinal fitness (Bearson and Bearson, 2008). Interestingly, there was no significant difference in swine fecal shedding between the *qseBC* double mutant and the wild-type *S. Typhimurium* (Figure 1). However, Merighi et al recently demonstrated that a *qseBC* double mutant of *S. Typhimurium* has a two-day delay in mouse mortality compared to the wild-type strain (Merighi et al., 2009).
Unfortunately, Merighi et al did not investigate the pathogenesis of a qseC mutant of S. Typhimurium in the mouse model to facilitate a comparison of the data between the swine and mouse models. Transcriptional analysis indicated that multiple S. Typhimurium genes are up-regulated in the presence of the QseB response regulator but in the absence of the QseC sensor kinase (QseB' QseC') compared to the presence of both QseB and QseC (QseB' QseC) (Merighi et al., 2009). This suggests that the QseC sensor kinase is required to prevent unwarranted gene expression by the QseB response regulator. The increased expression of certain genes in the qseC mutant may account for its decreased fitness in the swine gastrointestinal tract. Thus, although colonization fitness was similar for both the qseBC double mutant and wild-type S. Typhimurium in swine, optimal expression of QseBC-regulated genes is required for systemic infection in mice.

![Graph showing average CFU/g feces over time](image)

Figure 1. Decreased swine fecal shedding of a S. Typhimurium qseC mutant compared to qseBC double mutant and wild-type strain following intranasal inoculation of 7-week old piglets (n=5 per strain) with ~1 x 10^7 CFU.

Iron is an essential element for bacterial growth but is toxic at higher concentrations, therefore the acquisition of iron is tightly controlled by the bacterial cell. Iron availability is limited in certain environments including the host, and bacteria utilize iron chelators termed siderophores to bind iron for transport into the bacterial cell. The fepA, iroN, and cirA genes encode outer membrane proteins for the transport of the siderophores, enterochelin (FepA), salmochelin (IroN) and their breakdown products (CirA) across the bacterial outer membrane. These iron acquisition genes were identified in an investigation of gene products required for norepinephrine-enhanced growth of S. Typhimurium in a serum containing medium (Bearson et al., 2008; Rabsch et al., 2003). A fepA iroN cirA mutant of S. Typhimurium was demonstrated in the mouse model of typhoid fever to be significantly attenuated (Rabsch et al., 2003). The fepA iroN cirA mutant also elicited significant protection against subsequent challenge with wild-type S. Typhimurium in mice (Williams et al., 2006). Additionally, an iroN mutant was demonstrated to have decreased competitive fitness in the colon of streptomycin-pretreated mice compared to wild-type S. Typhimurium (Raffatellu et al., 2009). In contrast to results in the mouse model, no significant difference in gastrointestinal colonization or fecal shedding was found comparing a S. Typhimurium fepA iroN cirA mutant to the wild-type strain in the natural swine host (Bearson et al., 2008). This suggests that fepA, iroN, and cirA gene products are not required for colonization of the swine gastrointestinal tract but are required by S. Typhimurium for systemic disease, indicating that extraintestinal virulence requires salmochelin for iron acquisition. Although we did not assess the colonization of a strain containing an iroN mutation by itself, our data with the fepA iroN cirA mutant suggests that this mutant would be able to colonize the swine gastrointestinal tract potentially due to
production of other siderophores by commensal microorganisms within the swine gastrointestinal tract. Therefore, redundancies in bacterial iron uptake and utilization systems including other siderophore transport mechanisms promote the growth of the *fepA iroN cirA* mutant in the swine gastrointestinal tract, whereas streptomycin-pretreatment in mice decreased the commensal microorganisms, forcing the *iroN* mutant to rely on endogenously-produced siderophores (salmochelin) which it was unable to utilize due to mutation of *iroN*.

**Conclusion**

Much of the scientific knowledge concerning *Salmonella* pathogenesis is based on in vitro cell culture and the mouse model of typhoid fever. The pathogenic potential of *S. Typhimurium* is a complex interaction between multiple microbial components and the variable environmental conditions within the host which requires optimal regulation of the bacterial genetic systems within the various in vivo environments. Our research demonstrates that there are differences in the pathogenesis and colonization of *S. Typhimurium* when comparing systemic disease in mice and gastrointestinal colonization/enteric disease in swine. Therefore, the evaluation of interventions to enhance food safety by decreasing *Salmonella* in swine and potentially other livestock can not be based solely on the mouse model of typhoid fever. Although pathogen attenuation in a systemic disease model can be an initial guide for potential intervention strategies, a natural host model system that evaluates the gastrointestinal colonization and enteric disease of *Salmonella* is required to validate efficacy and enhance pork safety.

**References**


MERIGHI, M., et al., 2009. Genome-wide analysis of the PreA/PreB (QseB/QseC) regulon of *Salmonella enterica* serovar Typhimurium. BMC Microbiol. 9, 42.


