HtpG and STM4067 contribute to long-term Salmonella Typhimurium persistence in pigs

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Introduction

Persistent Salmonella Typhimurium infections in pigs often result in asymptomatic carrier pigs and are a major concern for food safety and human health. Tonsils and lymph nodes play a key role in the persistence of Salmonella Typhimurium in pigs, but very little is known about the underlying mechanisms. After bacterial invasion in pigs, the porcine immune system will respond to clear the Salmonella infection and bacterial survival strategies for (long-)term persistence will become important. For the identification of Salmonella Typhimurium genes specifically induced in tonsils and lymph nodes at 3 weeks post inoculation, a genome-wide screening method was performed using in vitro expression technology (IVET)1.

Materials and Methods

We used a spontaneous nalidixic acid resistant derivative of a virulent wild type Salmonella Typhimurium (WT) strain isolated from a pig tonsillar sample. For proper use in the IVET protocol, we verified if a part of Salmonella Typhimurium mutants2 was significantly impaired in comparison to the wild type strain in an in vitro mixed infection experiment. We constructed an IVET library as described earlier for Salmonella Enteritidis1, composed of approximately 12,000 different transformants, representing the major part of the Salmonella Typhimurium genome. For IVET selection in pigs, 9 pigs were orally inoculated with 10^8 colony forming units (CFU) of the IVET library. Three weeks after inoculation, pigs were euthanized and tonsils and lymph nodes were collected, processed and plated on selective MacConkey agar. White colonies, representing transformants with in vitro but not in vivo induced genes, were collected and the IVET fusions were sequenced as described before3. Identification of the sequence of the cloned promoter was done by BLAST analysis. Three mutants in in vitro induced genes were constructed2 (ΔhtpG, ΔhtpG and ΔSTM4067) and used in a subsequent mixed infection experiment. Three groups of 6 piglets were inoculated with 2 x 10^7 CFU of the WT and 2 x 10^7 CFU of 1 of the 3 constructed mutants. After euthanization, tonsils, ileum (+contents), ileocaecal lymph nodes, caecum (+contents) and faeces were analyzed for the number of Salmonella Typhimurium bacteria.

Discussion

Using IVET, Huang et al. already identified several Salmonella Typhimurium genes expressed in porcine tonsils at 2 days post inoculation4. These genes differ from the genes that we were able to identify at 3 weeks post inoculation (except for rpoN), suggesting that different sets of Salmonella genes play a role in short- and long-term persistence in pigs. Furthermore, we were able to show a role for Salmonella Typhimurium htpG (encoding a heat-shock protein) and STM4067 (encoding a protein with an unknown function) in long-term persistence in the porcine intestines and lymph nodes, although their exact role remains to be clarified.

Results

From the IVET selection experiment, 19 and 24 in vivo induced genes were identified in the tonsils and lymph nodes respectively (summarized in Table I). One known virulence gene (ΔsifB) and 2 genes encoding factors playing a role in Salmonella stress responses (htpG and dnaK) were identified. The majority of the identified genes plays a role in Salmonella metabolism or exert a yet unknown function in Salmonella persistence in pigs. From the 3 genes examined in the subsequent mixed infection experiment, ΔhtpG and ΔSTM4067 were identified in colonization of the intestines and lymph nodes of pigs, compared to the wild type; this was not the case for ΔsifB. Furthermore, none of the 3 examined Salmonella genes played a role in Salmonella persistence of porcine tonsils (Figure 1).

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