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Coated fatty acids alter virulence properties of *Salmonella* Typhimurium and decrease intestinal colonization of pigs

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Abstract

Salmonella Typhimurium infections in pigs are a major source of human foodborne salmonellosis. To reduce the number of infected pigs, acidification of feed or drinking water is a common practice. The aim of the present study was to determine whether some frequently used short- (SCFA) and medium-chain fatty acids (MCFA) are able to alter virulence gene expression and to decrease *Salmonella* Typhimurium colonization and shedding in pigs using well established and controlled *in vitro* and *in vivo* assays. Minimal inhibitory concentrations (MIC) of 4 SCFA (formic acid, acetic acid, propionic acid and butyric acid) and 2 MCFA (caproic and caprylic acid) were determined using 54 porcine *Salmonella* Typhimurium field strains. MIC values increased at increasing pH-values and were two to eight times lower for MCFA than for SCFA. Expression of virulence gene *fimA* was significantly lower when bacteria were grown in LB-broth supplemented with sub-MIC concentrations of caproic or caprylic acid (2 mM). Expression of *hilA* and invasion in porcine intestinal epithelial cells was significantly lower when bacteria were grown in LB-broth containing sub-MIC concentrations of butyric acid or propionic acid (10 mM) and caproic or caprylic acid (2 mM). When given as feed supplement to pigs experimentally infected with *Salmonella* Typhimurium, coated butyric acid decreased the levels of faecal shedding and intestinal colonization, but had no influence on the colonization of tonsils, spleen and liver. Uncoated fatty acids, however, did not influence fecal shedding, intestinal or tonsillar colonization in pigs. In conclusion, supplementing feed with certain coated fatty acids, such as butyric acid, may help to reduce the *Salmonella* load in pigs.

Keywords: *Salmonella* Typhimurium; Pig; SCFA; MCFA; Butyric acid

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1. Introduction

In Europe, *Salmonella* Typhimurium is by far the dominant serovar isolated from pigs (EFSA, 2008). In most cases, *Salmonella* Typhimurium will subclinically colonize the pigs, without causing obvious symptoms. These carrier pigs are a vast reservoir of *Salmonella* and pose a major threat to animal and human health (Boyen et al., 2008).

The battle against non-typhoidal *Salmonella* infections in pigs requires a strategic implementation of different approaches across the pork production and processing chains (Ojha and Kostrzynska, 2007). In addition to general hygiene and biosecurity measures, the supplementation of feed with acidic compounds has been proposed as a possible tool to combat *Salmonella* in pigs (Creus et al., 2007). Currently, short- (SCFA) and medium-chain fatty acids (MCFA) are commonly used in the poultry industry for this purpose (Van Immerseel et al., 2006). Apart from their antimicrobial actions at high concentrations, even low concentrations of SCFA and MCFA can decrease intestinal colonization by *Salmonella* Enteritidis in poultry, mediated by their influence on virulence gene expression (Van Immerseel et al., 2004, 2005; Gantois et al., 2006).

It was the aim of the present study to evaluate the usefulness of SCFA and MCFA in controlling *Salmonella* infections in pigs. Minimal inhibitory concentrations (MIC) of 4 SCFA and 2 MCFA for 54 *Salmonella* Typhimurium strains were determined. The influence of sub-MIC concentrations of these acids on virulence gene expression and invasive capacities of *Salmonella* Typhimurium was evaluated. Finally, the efficacy of coated as well as uncoated fatty acids in reducing the early colonization of piglets inoculated with *Salmonella* Typhimurium was assessed in two *in vivo* trials.

2. Materials and methods

2.1. Bacterial strains

Salmonella Typhimurium strain 112910a (DT 120/ad) was used in all *in vitro* experiments and its invasive nalidixic acid-resistant derivative was used in the *in vivo* trial. Strain 112910a was isolated from a pig stool sample and persists in tonsils, intestines and gut-associated lymphoid tissue (GALT) of experimentally infected pigs during at least 28 days (Boyen et al., in press).

Fifty-four independent *Salmonella* Typhimurium strains, isolated from pigs in Belgian slaughterhouses and farms, were used to perform minimal inhibitory concentration assays.

2.2. Minimal inhibitory concentrations of fatty acids

Minimal inhibitory concentrations were determined for SCFA and MCFA at pH 4, 5 and 6, using HCl or NaOH to obtain the different pH-values. Formic acid (C_1), acetic acid (C_2), propionic acid (C_3), butyric acid (C_4), caproic acid (C_6) and caprylic acid (C_8) (all products from Sigma, St. Louis, Mo.) were tested after serial two-fold dilutions in 96-well microplate in LB-broth ranging from 0.0391 to 2560 mM. Bacteria were grown for 18 h in 5 ml Luria-Bertani broth (LB) at 37 °C. Five microliters of this suspension was inoculated in 195 μl medium in each microwell plate. These suspensions were incubated for 20 h at 37 °C after which bacterial growth was assessed.

2.3. Construction of the transcriptional fusions

The pCS26 plasmid was used for the construction of transcriptional fusions between the promoter region of *fimA* (Althouse et al., 2003) and the *luxCDABE* operon as described before for the *hilA* promoter region (Van Immerseel et al., 2004). In short, the predicted promoter sequence of *fimA* was amplified by PCR and cloned into the pCS26 plasmid. Primers used for amplifying the promoter sequence of *fimA* were NNNNCTCGAGTGGCTATGGTTACCGTAATC (forward primer) and NNNNGGATCCAGGCTGCATTAACCAGTTTACC (reverse primer). Both the pCS26 plasmid and the amplification product containing the promoter sequence were digested and ligated. The ligation mixture was used for electroporation of *Salmonella* Typhimurium strain 112910a and kanamycin-resistant colonies (selection marker of pCS26) were tested for the promoter-plasmid junction by PCR. The sequence of the promoter-plasmid junction was confirmed by DNA sequencing.