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Salmonella serovar-specific interaction with jejunal epithelial cells

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ABSTRACT

Gut is often a receptacle for many different pathogens in feed and/or the environment, such as *Salmonella* spp. The current knowledge about pathogenicity of *Salmonella* is restricted to few serotypes, whereas other important ones like S. Coeln, S. Thompson, S. Veneziana, have not been investigated yet in human and animal models. Therefore, the aim of our work was to verify the ability of widespread environmental *Salmonella* strains to penetrate and modulate innate immunity in pig intestinal IPEC-J2 cells.

Our results outline the different ability of *Salmonella* strains to modulate innate immunity; the expression of the IFN-β gene was increased by S. Typhimurium, S. Ablogame and S. Diarizonae 2, that also caused an inflammatory response in terms of Interleukin (IL)-1β and/or IL-8 gene espression. In particular, IL-8 gene expression and protein release were significantly modulated by 5 *Salmonella* strains out of 7. Interestingly, S. Typhimurium, S. Coeln and S. Thompson strains, characterized by a peculiar ability to penetrate into IPEC-J2 cells, up-regulated both IL-8 and TNF-α gene expression. Accordingly, blocking IL-8 was shown to decrease the penetration of S. Typhimurium. On the contrary, S. Diarizonae strain 1, showing lesser invasion of IPEC-J2 cells, down-regulated the p38-MAPK pathway, and it did not induce an inflammatory response. Our results confirm that IPEC-J2 cells are a useful model to evaluate host-gut pathogen interaction and indicate IL-8 and TNF-α as possible predictive markers of invasiveness of *Salmonella* strains in enterocytes.

1. Introduction

The gut is often a receptacle for numerous pathogens encountered in feed or in the environment; among these, some bacterial species may play an important role as both animal pathogens and source of contamination of meat and other products of animal origin. This is the case of the *Salmonella* genus, which poses a substantial hazard to humans as food-borne pathogens.

The *Salmonella* genus belongs to the family Enterobacteriaceae; it includes gram-negative, rod-shaped (0.7–1.5 × 2.5 μm), oxidase negative, catalase positive, facultative anaerobic bacteria, able to exploit citrate as the sole carbon source. To date, only two species of the genus *Salmonella* are recognized, i.e. S. enterica and S. bongori, whereas more than 2500 serotypes are identified on the basis of somatic (A–B–C_1–C_2–D–E_1) and flagellar antigens (H1–H2), located in the cell wall (Kim et al., 2006), and of biochemical tests (Popoff et al., 2001). Currently, *Salmonella* is divided into two main groups, typhoid (TS) and non-typhoid (NTS), respectively, but from an epidemiological point of view *Salmonella* can be also divided into three further groups: human-adapted like S. Typhi, S. Paratyphi A, B and C; *Salmonella* adapted to other animal species (e.g. S. Gallinarum, S. Abortus equi and S. Choleraesuis) and non-adapted like S. Typhimurium and S. Enteritidis. The latter group includes serotypes present in animal and human reservoirs and are widespread in the environment. Many serotypes identified to date, like S. Coeln, S. Goldcost, S. Kottbus, S. Stourbridge, S. Thompson, S. Veneziana have been sustained for their pathogenicity in humans. However, since outbreaks of food poisoning by these serotypes have not been investigated in the last twenty years, it is necessary to clarify their pathogenic potential (Dionisio et al., 2001; Espie and Vaillant, 2005; Palmera-Suarez et al., 2007; Graziani et al., 2011; Scavia et al., 2013). In recent years, growing attention has been directed on two NTS serotypes, S. Typhimurium and S. Enteritidis, which has led to the enactment of specific legislation in Europe (**Directive 2003/99/EC**, Decision 2013/652/EU, **Regulation EC No 2160/2003**). This was related to the higher prevalence of isolates, 22.1% and 41.3% for S. Typhimurium and S. Enteritidis, respectively, in human cases found in Europe (Hugas and Beloeil, 2014), as well as to a higher prevalence of multi-drug resistance observed in S. Typhimurium strains (Chiu et al., 2006).

Also, important studies highlighted the molecular basis of

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Table 1
Oligonucleotide Primer Sequences for Evagreen Quantitative Reverse Transcription Real-Time Polymerase Chain Reaction Amplification of Porcine Genes.

Gene	Protein	Primers	Accession number	Bp
pB2M	β2 Microglobulin	F:5 –CGCCCCAGATTGAAATTGATTTGC-3 R:5 –GCTATACTGATCCACAGCGTTAGG-3	NM_213978.1	139
IL-1β	Interleukin-1β	F:5 –AATTCGAGTCTGCCCTGTACCC-3 R:5 –TGGTGAAGTCGGTTATATCTTGGC-3	NM_001005149	110
IL-4	Interleukin-4	F:5 –GGACACAAGTGCGACATCA-3 R:5 –GCACGTGTGGTGTCTGTA-3	NM_214123.1	185
IL-6	Interleukin-6	F:5 –TGGCTACTGCCTTCCCTACC −3 R:5 –CAGAGATTTTGCCGAGGATG-3	NM_214399	131
IL-8	Interleukin-8	F:5 –TTCGATGCCAGTGCATAAATA −3 R:5 –CTGTACAACCTTCTGCACCCA-3	NM_213867.1	175
TNF-α	Tumor Necrosis Factor-α	F:5 –TGCCTACTGCACTTCGAGGTTATC-3 R:5 –GTGGGCGACGGGCTTATCTG-3	NM_214022	125
IFN-β	Interferon-β	F:5 –AGTTGCCTGGGACTCCTCAA-3 R:5 –CCTCAGGGACCTCAAAGTTCAT-3	JF906509.1	59
βD-1	β-defensin-1	F:5 –CTGTTAGCTGCTTAAGGAATAAAGGC-3 R:5 –TGCCACAGGTGCCGATCT-3	NM_213838	80
βD-2	β-defensin-2	F:5 –CCAGAGGTCCGACCACTACA-3 R:5 –GGTCCCTTCAATCCTGTTGAA-3	NM_214442	87
βD-3	β-defensin-3	F:5 –CTTCCTATCCAGTCTCAGTGTTCTGC-3 R:5 –GGCTTCTGTAGACTTCAAGGAGACAT-3	NM_214444.1	308
βD-4	β-defensin-4	F:5 –GTGGCTTGGATTTGAGGAGAGAGT-3 R:5 –AGTGATACACAGGCCTGGAAGGAT-3	NM_214443.1	232
IL-18	Interleukin-18	F:5′–CGTGTTTGAGGATATGCCTGATT-3′ R:5′–TGGTTACTGCCAGACCTCTAGTGA-3′	AF191088.1	106
SOCS-1	Suppressor of cytokine signaling 1	F:5′–TTCTTCGCCCTCAGTGTGAA-3′ R:5′–GGCCTGGAAGTGCACGC-3′	NM_001204768.1	62
TLR-4	Toll-like Receptor 4	F:5′–TGGCAGTTTCTGAGGAGTCATG-3′ R:5′–CCGCAGCAGGGACTTCTC-3′	AB188301.2	71
TLR-5	Toll-like Receptor 5	F:5′ –CCAGCT GTA TCA GGG AGC TT −3′ R:5′ –TCA AAG ATC CTG ACC ATC ACA −3′	NC_010452.3	59
MYD88	Myeloid differentiation primary response gene 88	F:5′ –GCA GCT GGA ACA GAC CAA CT-3′ R:5′ –GTG CCA GGC AGG ACA TCT-3′	NM_001099923.1	62
NF-kB1	Nuclear Factor kB	F:5′–CCCATGTAGACAGCACCACCTATGAT-3′ R:5′–ACAGAGGCTCAAAGTTCTCCACCA-3′	NM_001048232	131
NF-kB/p65	RELA protein	F:5′ –CGAGAGGAGCACGGATACCA-3′ R:5′ –GCCCCGTGTAGCCATTGA-3′	FN999988.1	61
JNK 1	Mitogen-activated protein kinase 8 (MAPK8)	F:5′ –TGCTTTGTGGAATCAAGCAC-3′ R:5′ –TGGGCTTTAAGTCCC-3′	XR_001301066.1	59
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	F:5′ –AACTCCTAGGACCTGGTGTGAA-3′ R:5′ –CGCTCCCTCTCCTTACTGATAA-3′	XM_005668829.2	192
P38α	Mitogen-activated protein kinase 14 (MAPK14)	F:5′ –TGCAAGGTCTCTGGAGGAAT-3′ 5′ –CTGAACGTGGTCATCCGTAA-3′	XM_013977842.1	109
CD14	CD14 molecule	F:5′ –TGCCAAATAGACGACGAAGA-3′ R:5′ –ACGACACATTACGGAGTCTGA-3′	EF626695.1	364
MD2	Lymphocyte antigen 96	F:5′ –TGCAATTCCTCTGATGCAAG-3′ R:5′ –CCACCATATTCTCGGCAAAT-3′	NM_001104956.1	207

pathogenesis of well-known *Salmonella* serotypes (Que et al., 2013; Spano, 2016). In particular, these studies revealed how these pathogens exploit inflammation to their own advantage to pass through the intestinal barrier and spread within the host (Elewaut et al., 1999; Gewirtz et al., 2000). The studies on NTS pathogenicity have focused more on S. Typhimurium, whereas other environmental serotypes, widely found in earlier foodborne poisoning cases, were neglected, so that studies of pathogenic capacity are lacking.

Studies of the pathogenic capacity of *Salmonella* spp. could conveniently be based on IPEC-J2 cells. These are an established cell line obtained from the jejunum of a piglet less than 12-h old (Brosnahan and Brown, 2012), and together with IPI-2I cells are a substrate of reference for studies on innate immunity in the gut. In particular, IPEC-J2 cells were shown to have ideal characteristics in studies on the host/gut pathogen interaction (Schierack et al., 2006). Therefore, IPEC-J2 cells have been widely employed to model zoonotic enteric infections (Scharek and Tedin, 2007; Brosnahan and Brown, 2012).

The aim of our work was to verify the ability of different environmental *Salmonella* strains to penetrate into IPEC-J2 cells and to modulate gene expression and release of important molecules involved in the innate immune response.

2. Materials and methods

2.1. Bacterial strains

In our study, seven field isolates of *Salmonella* enterica (resistant to Trisulfapyrimidines –Sulfadiazine, Sulfamethazine and Sulfamerazine) were investigated: S. Coeln, S. Veneziana, S. Ablogame, S. Diarizonae strain 1, S. Thompson, S. Thyphimurium and S. Diarizonae strain 2, all isolated from wild boar livers. Bacteria were stored at −80 °C in 50% (v/v) glycerol/Brain Heart Infusion (BHI) broth and then grown overnight in Luria-Bertani (LB) medium at 37 °C. Spectrophotometric measures indicated that this treatment was sufficient to reach the stationary growth phase. Then, overnight cultures (200 µl) were inoculated into fresh LB medium (10 ml) and incubated for 2–3 h at 37 °C to obtain mid-log phase cultures. Then, each strain was pelleted and resuspended at 10^8 CFU/ml in MEM/F12 cell culture medium.

2.2. Cells

IPEC-J2 cells (porcine intestinal epithelial cells, IZSLER Cell Bank code BS CL 205) were grown in a mixture of Minimum Essential Medium (MEM) and Ham's F12 medium (1:1) enriched with fetal calf