This is a pre- or post-print of an article published in

Uliczka, F., Dersch, P.

**Unique virulence properties of *Yersinia enterocolitica*
O:3**

**(2012) Advances in Experimental Medicine and Biology,
954, pp. 281-287.**

Unique virulence properties of *Yersinia enterocolitica* O:3

Frank Uliczka^{1,2} and Petra Dersch^{1,2*}

¹Department of Molecular Infection Biology, Helmholtz Center for Infection Research, Braunschweig, Germany, ²Department of Microbiology, Technical University Braunschweig, Germany

* corresponding author: petra.dersch@helmholtz-hzi.de

1. Introduction

Pathogenic *Y. enterocolitica* strains of biotype 1B, 2, 3 or 4 are harmful to humans and animals (Iteman *et al.*, 1996). Due to the presence of a high pathogenicity island in biotype 1B, these strains are considered to be highly pathogenic (Carniel, 2001). The most common and frequently isolated *Y. enterocolitica* bio-serotypes are 1B/O:8, 2/O:5,27, 2/O:9, 3/O:3 and 4/O:3 (Bottone, 1999). Most of the pathogenic *Y. enterocolitica* strains in Europe are classified as bioserotype 4/O:3 strains (YeO:3), whereas strains classified as bioserotype 1B/O:8 (YeO:8) are more common in North America. However, their relevance decreased and more occasional outbreaks of serogroup O:3 strains have been reported over the last 30 years (Bissett *et al.*, 1990; Tauxe, 2002; Tauxe *et al.*, 1987). YeO:3 strains commonly colonize the lymphoid tissues of the oropharynx, nasopharynx and the intestine of pigs, which are considered to be their natural reservoir. Pigs are usually asymptomatic carriers without clinical signs in the digestive tract or in the tonsils and represent a substantial disease-causing potential for humans. As a result, outbreaks of Yersiniosis are mostly associated with the consumption of raw or incompletely cooked meat (de Boer & Nouws, 1991; Fredriksson-Ahomaa *et al.*, 2006; Tauxe *et al.*, 1987).

High prevalence of YeO:3 strains in pigs indicate serotype- and/or host-specific colonization strategies. However, very little is known about the molecular mechanisms that define bacterial colonization of these strains. Previous analyses showed that enteric *Yersinia* species (YeO:8 and *Y. pseudotuberculosis*) initiate infections of their mammalian hosts by tight attachment to mucosal surfaces in the intestine, which is frequently followed by invasion of subepithelial tissues. Multiple virulence factors of YeO:8 are known to promote efficient colonization of host tissues. These adhesion factors include the chromosomally encoded outer membrane protein invasin (Inv). Invasin is predominantly expressed at moderate temperature *in vitro*, and can be induced at 37°C under certain growth conditions (Pepe *et al.*, 1994). Invasin binds with high affinity to β_1 -integrins and induces uptake into host cells (Isberg *et al.*, 1987; Pepe & Miller, 1993). Another important adhesion factor is YadA which is only expressed at 37°C (El Tahir & Skurnik, 2001; Skurnik & Toivanen, 1992). YadA belongs to the family of trimeric autotransporter adhesins and was shown to bind with high affinity to collagen and laminin (Leo *et al.*,

2008; Skurnik & Toivanen, 1992). To investigate the colonization mechanisms of YeO:3 strains, we compared their cell adhesion and invasion properties with that of other *Y. enterocolitica* serotypes.

2. Serotype specific interaction of *Y. enterocolitica* with epithelial cells

Adhesion and invasion of different human and animal-derived *Y. enterocolitica* isolates were investigated using human, porcine and murine intestinal epithelial cells. All tested serotype O:5,27, O:8 and O:9 strains adhered and invaded efficiently into host cells independent of the origin of the cell line or the source of the isolate. In contrast, all serotype O:3 strains were only weakly adherent and unable to internalize into host cells, although high amounts of the primary internalization factor invasins were produced under standard growth conditions (Uliczka *et al.*, 2011).

Y. enterocolitica O:8 was known to be flagellated and motile when grown at moderate temperature (Young *et al.* 2000), and this also accounts for isolates of serotypes O:5,27, and O:9. However, none of the serotype O:3 strains were motile on swimming and swarming agar plates under various tested growth conditions. Interestingly, bacteria isolated from the intestine of BALB/c mice three days post infection expressed flagella immediately after recovery, but flagella synthesis was rapidly repressed during growth on agar plates (Uliczka *et al.*, 2011). It has previously been demonstrated that the motility of YeO:8 strain 8081v enhances bacteria-cell associations and stimulates host cell entry (Young *et al.* 2000). Although attachment of YeO:3 strains was slightly increased upon artificially induced host cell contact, the overall number of cell associated bacteria was still low compared to YeO:8.

Another conspicuous difference between the *Y. enterocolitica* serotypes resides in their LPS molecules composed of lipid A, the core and the O-antigen. Most O-specific polysaccharides from LPS of *Yersinia* form heteropolymers. In contrast, O-antigen of YeO:3 is composed of a homopolymer of 6-deoxy-L-altrose linked by α -1,2 glycosidic bonds. The O-antigen and the outer core are both connected to the inner core, resulting in the formation of a branched LPS molecule (Al-Hendy *et al.*, 1991; Bengoechea *et al.*, 2004; Brubaker, 1991). An interesting feature of *Y. enterocolitica* O antigen is that its production is tightly controlled in response to temperature. *Y. enterocolitica* produce O-antigen in abundance at 22-25°C, but only very low amounts can be detected at 37°C (Bengoechea *et al.*, 2004; 2002). This led to speculations, that the function of YeO:3 adhesion factors might be inhibited by the presence of the distinct YeO:3 O antigen. In fact, cell adhesion and invasion of a YeO:3 O-antigen mutant (YeO3-R2) was enhanced compared to the wild type, and could further be increased by an outer core knock-out mutation (YeO3-OCR) (Uliczka *et al.*, 2011). However, overall invasion of the mutants was still low compared to YeO:8. Hence, sterical hindrance by the unique LPS struc-

tures did not explain the profound differences of the cell binding properties of serotype O:3 strains.

3. Efficient host cell adhesion and invasion of YeO:3 is mediated by the cooperative action of invasin and YadA

Based on these results, also influence of the virulence plasmid-encoded adhesion factor YadA was studied. Since YadA is exclusively expressed at 37°C, cell adhesion and invasion assays were performed with YeO:3 and YeO:8 pregrown at host temperature. Both *Y. enterocolitica* serotypes adhered efficiently to human epithelial cells through YadA which was similarly expressed in all tested *Y. enterocolitica* O:3 and O:8 isolates.

In contrast, all YeO:3 strains, but none of the YeO:8 isolates were able to internalize into host cells. Cell invasion was shown to be solely mediated by the invasin protein, which was highly expressed at 37°C in all YeO:3 isolates, but not in YeO:8 strains (Uliczka *et al.*, 2011). Latter observation is consistent with previous studies, demonstrating that *inv* expression in YeO:8 strain 8081v is mostly expressed at moderate temperature, whereas only very low amounts of invasin were detectable at 37°C (Pepe *et al.*, 1994).

Although high amounts of invasin were expressed in YeO:3, cell binding and uptake was drastically reduced in a *yadA* deficient YeO:3 strain. Furthermore, overexpression of YadA induced adhesion and invasion also at 25°C even in the presence of inhibitory LPS structures (Uliczka *et al.*, 2011). This strongly suggests that YadA and invasin of YeO:3 act cooperatively, whereby YadA promotes efficient cell attachment allowing invasin to initiate the entry process (figure 1). These properties seem to be YeO:3 specific, as previous studies with YeO:8 and *Y. pseudotuberculosis* showed that invasin and YadA are differentially expressed and constitute independent colonization factors that are active at separate sites and/or during different stages of the infection (Eitel & Dersch, 2002; Pepe *et al.*, 1994; Skurnik & Toivanen, 1992).

Why distinct colonization strategies are used by the different enteropathogenic *Yersinia* strains is still unclear. However, it is tempting to speculate that interaction of the long, surface-extended YadA molecules initiates host cell contact of YeO:3 through binding to ECM-bound β_1 -integrins receptors. This interaction facilitates subsequent binding of the shorter invasin molecules to β_1 -integrins leading to the initiation of the internalization process. Why this mechanism is only restricted to pig-associated YeO:3 strains, which are less virulent for mice remains elusive. Previous studies have shown that invasin and YadA trigger the production of proinflammatory cytokines (e.g. IL-8) (Grassl *et al.*, 2003; Schmid *et al.*, 2004). Coexpression of both colonization factors may be tolerated by pigs, but could induce unfavorable immune responses in other hosts, e.g. rodents.

4. Invasin expression of YeO:3 is constitutive and depends on an IS1667-encoded promoter

High invasin expression at 37°C has been found in all available YeO:3 isolates collected from all over the world, indicating that this special property is important for multiplication and/or persistence in their favored hosts (pigs, boars). To unravel the underlying mechanism, we analyzed the invasin locus of the isolates and found that all strains contained an IS1667 insertion at position -143 of the *inv* promoter (figure 2). Expression of the *inv* gene was induced in YeO:3, but not in YeO:8 or a IS1667 deletion variant of YeO:3 (YeO:3*inv*ΔIS1667), indicating that increased activity of the *inv* promoter in YeO:3 is caused by the IS1667 insertion (Uliczka *et al.*, 2011).

A detailed promoter analysis demonstrated that an IS1667-encoded, σ^{70} -dependent promoter (P_{IS1667}) oriented outward of the IS element is responsible for the constitutive activation of *inv* transcription. Importance of P_{IS1667}-driven *inv* expression for virulence was further demonstrated by coinfection experiments in which equal amounts of YeO:3 and the isogenic YeO:3*inv*ΔIS1667 mutant was orally applied to BALB/c mice. Colonization of the Peyer's patches and the mesenteric lymph nodes by YeO:3*inv*ΔIS1667 was significantly reduced and deeper tissues were only sporadically reached by the mutant in contrast to wild type strain (Uliczka *et al.*, 2011). This strongly indicates, that high amounts of invasin expressed by YeO:3 is advantageous for the progress of the infection in mice. Interestingly, expression of *inv* seems less important for YeO:8. A YeO:8 *inv* mutant was still able to colonize the lymphatic tissues and organs with similar LD₅₀ values; only progression of the infection was slightly delayed (Pepe & Miller, 1993; Revell & Miller, 2000). It is most likely that other colonization factors support invasin function in this serotype. In fact, computational analysis revealed that several other genes of invasin- and YadA-type molecules are encoded by YeO:8 8081v (Thomson *et al.*, 2006).

5. RovA and H-NS interact preferentially with IS1667-sequences and influence *inv* transcription in YeO:3

Previous analysis of *inv* transcription in YeO:8 8081v revealed several proteins implicated in the control of *inv* transcription. The histone-like protein H-NS was shown to silence the *inv* promoter in a complex with YmoA, a member of the Hha regulator family (Ellison *et al.*, 2004; 2003; Lawrenz & Miller, 2007). H-NS is necessary for the nucleoid packaging of the bacterial chromosome and regulates a variety of genes depending on temperature (Atlung & Ingmer, 1997; Ghosh *et al.*, 2006; Müller *et al.*, 2006). Gene silencing mediated by H-NS is caused by preferentially binding to AT-rich DNA sequences displaying intrinsic curvatures (Olivares-Zavaleta *et al.*, 2006; Rimsky, 2004; Schröder & Wagner, 2002). Acti-

vation of *inv* in enteropathogenic *Yersinia* spp. is mediated by the MarR-type transcriptional factor RovA (Revell & Miller, 2000; Nagel *et al.* 2001). The RovA and H-NS binding sites are superimposed in the *inv* regulatory region, and RovA is able to alleviate H-NS repression at moderate temperatures in *Y. enterocolitica* YeO:8 and *Y. pseudotuberculosis* (Ellison *et al.*, 2004; Heroven *et al.*, 2004).

Two RovA binding sites have been identified upstream of the *inv* promoter (Heroven *et al.*, 2004). The insertion of the IS1667 elements in the YeO:3 strains disrupted the distal RovA binding site and moved part of this region upstream of the IS element (figure 2). Expression analysis of this promoter variant clearly showed that *inv* transcription by the IS1667 promoter is still RovA dependent despite the destruction and separation of its binding sites. Purified RovA still bound to the partial RovA binding site upstream of the IS insertion, but deletion of this region had no effect on the transcription of *inv*. In addition and more important, RovA interacted specifically with IS1667 sequences downstream of the P_{IS1667} promoter, replacing parts of the original RovA binding region (figure 2). As also H-NS was found to bind preferentially to this P_{IS1667}-encoded binding site, it is postulated that RovA is required to alleviate H-NS-mediated repression of the constitutive IS1667 promoter driving *inv* expression (Uliczka *et al.*, 2011).

6. Role of RovA for virulence of YeO:3

Analysis of RovA synthesis in YeO:3 strains further revealed that *rovA* transcription is not autoregulated and only weakly controlled by temperature. Moreover, significantly higher amounts of RovA were identified in YeO:3 strains at 25°C and 37°C. This result was unexpected, as it was recently shown that RovA synthesis of *Y. pseudotuberculosis* is positively autoregulated and subjected to temperature regulation on the post-transcriptional level (Herbst *et al.*, 2009). RovA acts as an intrinsic thermometer. A temperature upshift from 25°C to 37°C (e.g. upon uptake by the human host) induces reversible conformational changes. These alterations reduce the DNA-binding capacity of RovA and render the regulator more susceptible to proteolysis by the Lon protease (Herbst *et al.*, 2009). As a consequence, RovA of *Y. pseudotuberculosis* is rapidly degraded at 37°C and less able to induce its own transcription due to a lower DNA binding ability. Investigations of RovA of YeO:8 (RovA_{O:8}) demonstrated similar thermosensing and degradation features (Uliczka *et al.*, 2011). They also resulted in rapid elimination of the regulator at 37°C, despite the fact that autoregulation of *rovA* in YeO:8 is less pronounced (Lawrenz & Miller, 2007).

Sequence analysis of the YeO:3 *rovA* locus revealed a single amino acid substitution in 45 of 48 analyzed YeO:3 isolates, leading to a proline to serine exchange at position 98. A detailed comparative analysis showed that the stability of the RovA_{S98} variant was significantly increased in comparison to RovA_{P98}, whereas the thermosensing and the DNA-binding abilities remained unaffected. Stabiliza-

tion of RovA alone accomplishes RovA production at 37°C and this is sufficient to displace H-NS from the *inv* promoter and the 3' end of the IS1667 element.

To investigate whether stabilization of RovA has an influence on the infection process, we performed coinfection experiments with equal amounts of wild-type YeO:3 and an isogenic mutant expressing the RovA_{p98} variant. Strikingly, significantly more bacteria could be isolated from lymphatic tissues and organs of the YeO:3 mutant expressing the unstable RovA_{p98} derivative (Uliczka *et al.*, 2011). This strongly indicated that high RovA levels are disadvantageous for YeO:3 during the infection of mice.

Previous work with YeO:8 and *Y. pseudotuberculosis* demonstrated that RovA is crucial for virulence (Heroven & Dersch, 2006; Revell & Miller, 2000). RovA-deficient strains were severely attenuated and significantly less bacteria could be recovered from the lymphatic tissues and organs after oral infection. Microarrays defining the RovA regulon showed that multiple genes are up- or downregulated by RovA. Among the repressed genes are metabolic genes e.g. amino acid transporters (Cathelyn *et al.*, 2007) and their upregulation during infection may enhance the fitness and persistence of the bacteria in mice. YeO:8 is highly virulent for rodents. In contrast, YeO:3 is less virulent for mice, but seems optimally adapted for colonizing pigs and boars. As the body temperature of swine varies between 39°C-40°C, a more stable RovA variant would adapt the control circuits and allow expression of RovA-dependent genes at higher temperatures.

7. Conclusions

Y. enterocolitica serotype O:3 frequently found in pigs is the most frequent cause of yersiniosis in Europe and seems to replace serotype O:8 as the predominant subtype in Northern America. Newly emerging variants of the same species are often characterized by small variations of their colonization factors, which allow adaptation to different or varying hosts or niches within hosts. By the comparison of YeO:8 and YeO:3 small genetic variations were identified between both serotypes which lead to profound changes of the virulence gene expression pattern. An IS insertion causes a high and constitutive expression of the primary adhesion and invasion factor invasins, and a base pair substitution results in the production of a more stable variant of the virulence regulator RovA in YeO:3. Both changes have a significant effect on YeO:3 virulence in mice and illustrated that an adaptation of the expression level of gene subsets allowed a fine-tuned readjustment of virulence-associated processes to their requirements in swine with higher body temperature.

8. Acknowledgments

We thank Dr. Ann Kathrin Heroven for critical reading of the manuscript. Work described in this study was supported by the BMBF (Consortium FBI-Zoo), der Deutschen Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

References

- Al-Hendy, A., Toivanen, P. & Skurnik, M. (1991). The effect of growth temperature on the biosynthesis of *Yersinia enterocolitica* O:3 lipopolysaccharide: temperature regulates the transcription of the *rfb* but not of the *rfa* region. *Microbial Pathogenesis* **10**, 81-6.
- Atlung, T. & Ingmer, H. (1997). H-NS: a modulator of environmentally regulated gene expression. *Molecular Microbiology* **24**, 7-17.
- Bengoechea, J. A., Zhang, L., Toivanen, P. & Skurnik, M. (2002). Regulatory network of lipopolysaccharide O-antigen biosynthesis in *Yersinia enterocolitica* includes cell envelope-dependent signals. *Molecular Microbiology* **44**, 1045-62.
- Bengoechea, J. A., Najdenski, H. & Skurnik, M. (2004). Lipopolysaccharide O antigen status of *Yersinia enterocolitica* O:8 is essential for virulence and absence of O antigen affects the expression of other *Yersinia* virulence factors. *Molecular Microbiology* **52**, 451-69.
- Bissett, M. L., Powers, C., Abbott, S. L. & Janda, J. M. (1990). Epidemiologic investigations of *Yersinia enterocolitica* and related species: sources, frequency, and serogroup distribution. *Journal of Clinical Microbiology* **28**, 910-2.
- de Boer, E. & Nouws, J. F. (1991). Slaughter pigs and pork as a source of human pathogenic *Yersinia enterocolitica*. *International journal of food microbiology* **12**, 375-8.
- Bottone, E. J. (1999). *Yersinia enterocolitica*: overview and epidemiologic correlates. *Microbes and Infection* **1**, 323-33.
- Brubaker, R. R. (1991). Factors promoting acute and chronic diseases caused by yersiniae. *Clinical Microbiology Reviews* **4**, 309-24.
- Carniel, E. (2001). The *Yersinia* high-pathogenicity island: an iron-uptake island. *Microbes and Infection* **3**, 561-9.
- Cathelyn, J. S., Ellison, D. W., Hinchliffe, S. J., Wren, B. W. & Miller, V. L. (2007). The *RovA* regulons of *Yersinia enterocolitica* and *Yersinia pestis* are distinct: evidence that many *RovA*-regulated genes were acquired more recently than the core genome. *Molecular Microbiology* **66**, 189-205.
- Eitel, J. & Dersch, P. (2002). The *YadA* protein of *Yersinia pseudotuberculosis* mediates high-efficiency uptake into human cells under environmental conditions in which *invasin* is repressed. *Infection and Immunity* **70**, 4880.
- El Tahir, Y. & Skurnik, M. (2001). *YadA*, the multifaceted *Yersinia* adhesin. *International Journal of Medical Microbiology : JMM* **291**, 209-18.
- Ellison, D. W., Young, B., Nelson, K. & Miller, V. L. (2003). *YmoA* negatively regulates expression of *invasin* from *Yersinia enterocolitica*. *Journal of Bacteriology* **185**, 7153-9.
- Ellison, D. W., Lawrenz, M. B. & Miller, V. L. (2004). *Invasin* and beyond: regulation of *Yersinia* virulence by *RovA*. *Trends in Microbiology* **12**, 296-300.
- Fredriksson-Ahomaa, M., Stolle, A., Siitonen, A. & Korkeala, H. (2006). Sporadic human *Yersinia enterocolitica* infections caused by bioserotype 4/O : 3 originate mainly from pigs. *Journal of Medical Microbiology* **55**, 747-9.
- Ghosh, A., Paul, K. & Chowdhury, R. (2006). Role of the histone-like nucleoid structuring protein in colonization, motility, and bile-dependent repression of virulence gene expression in *Vibrio cholerae*. *Infection and Immunity* **74**, 3060-4.
- Grassl, G. a, Bohn, E., Müller, Y., Bühler, O. T. & Autenrieth, I. B. (2003). Interaction of *Yersinia enterocolitica* with epithelial cells: *invasin* beyond invasion. *International Journal of Medical Microbiology* **293**, 41-54.
- Herbst, K., Bujara, M., Heroven, A. K., Opitz, W., Weichert, M., Zimmermann, A. & Dersch, P. (2009). Intrinsic thermal sensing controls proteolysis of *Yersinia* virulence regulator *RovA*. *PLoS Pathogens* **5**, e1000435.
- Heroven, A. K. & Dersch, P. (2006). *RovM*, a novel LysR-type regulator of the virulence activator gene *rovA*, controls cell invasion, virulence and motility of *Yersinia pseudotuberculosis*. *Molecular Microbiology* **62**, 1469-83.
- Heroven, A. K., Nagel, G., Tran, H. J., Parr, S. & Dersch, P. (2004). *RovA* is autoregulated and antagonizes H-NS-mediated silencing of *invasin* and *rovA* expression in *Yersinia pseudotuberculosis*. *Molecular Microbiology* **53**, 871-88.
- Isberg, R. R., Voorhis, D. L. & Falkow, S. (1987). Identification of *invasin*: a protein that allows enteric bacteria to penetrate cultured mammalian cells. *Cell* **50**, 769-78.

- Iteman, I., Guiyoule, A. & Carniel, E. (1996). Comparison of three molecular methods for typing and subtyping pathogenic *Yersinia enterocolitica* strains. *Journal of Medical Microbiology* **45**, 48-56.
- Lawrenz, M. B. & Miller, V. L. (2007). Comparative analysis of the regulation of *rovA* from the pathogenic yersiniae. *Journal of Bacteriology* **189**, 5963-75.
- Leo, J. C., Elovaara, H., Brodsky, B., Skurnik, M. & Goldman, A. (2008). The *Yersinia* adhesin YadA binds to a collagenous triple-helical conformation but without sequence specificity. *Protein Engineering, Design & Selection* **21**, 475-84.
- Müller, C. M., Dobrindt, U., Nagy, G., Emödy, L., Uhlin, B. E. & Hacker, J. (2006). Role of histone-like proteins H-NS and StpA in expression of virulence determinants of uropathogenic *Escherichia coli*. *Journal of Bacteriology* **188**, 5428-38.
- Nagel G., Lahrz A. & Dersch P. (2001). Environmental control of invasin expression in *Yersinia pseudotuberculosis* is mediated by regulation of RovA, a transcriptional activator of the SlyA/Hor family. *Molecular Microbiology* **41**, 1249-69.
- Olivares-Zavaleta, N., Jáuregui, R. & Merino, E. (2006). Genome analysis of *Escherichia coli* promoter sequences evidences that DNA static curvature plays a more important role in gene transcription than has previously been anticipated. *Genomics* **87**, 329-37.
- Pepe, J. C. & Miller, V. L. (1993). *Yersinia enterocolitica* invasin: a primary role in the initiation of infection. *Proc. Natl. Acad. Sci.* **90**, 6473-7.
- Pepe, J. C., Badger, J. L. & Miller, V. L. (1994). Growth phase and low pH affect the thermal regulation of the *Yersinia enterocolitica* *inv* gene. *Molecular Microbiology* **11**, 123-35.
- Revell, P. A. & Miller, V. L. (2000). A chromosomally encoded regulator is required for expression of the *Yersinia enterocolitica* *inv* gene and for virulence. *Molecular Microbiology* **35**, 677-85.
- Rimsky, S. (2004). Structure of the histone-like protein H-NS and its role in regulation and genome superstructure. *Current Opinion in Microbiology* **7**, 109-14.
- Schmid, Y., Grassl, G. A., Bühler, O. T., Skurnik, M., Autenrieth, I. B. & Bohn, E. (2004). *Yersinia enterocolitica* adhesin A induces production of interleukin-8 in epithelial cells. *Infection and Immunity* **72**, 6780-9.
- Schröder, O. & Wagner, R. (2002). The bacterial regulatory protein H-NS--a versatile modulator of nucleic acid structures. *Biological Chemistry* **383**, 945-60.
- Skurnik, M. & Toivanen, P. (1992). LcrF is the temperature-regulated activator of the *yadA* gene of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *Journal of Bacteriology* **174**, 2047-51.
- Tauxe, R. V. (2002). Emerging foodborne pathogens. *International Journal of Food Microbiology* **78**, 31-41.
- Tauxe, R., Wauters, G., Goossens, V., Noyen, R. V., Vandepitte, J., Martin, S., Mol, P. D. & Thiers, G. (1987). *Yersinia enterocolitica* infections and pork: the missing link. *The Lancet* **329**, 1129-1132.
- Thomson, N. R., Howard, S., Wren, B. W., Holden, M. T. G., Crossman, L., Challis, G. L., Churcher, C., Mungall, K., Brooks, K., & other authors. (2006). The complete genome sequence and comparative genome analysis of the high pathogenicity *Yersinia enterocolitica* strain 8081. *PLoS Genetics* **2**, e206.
- Uliczka, F., Pisano, F., Schaake, J., Stolz, T., Rohde, M., Fruth, A., Strauch, E., Skurnik, M., Batzilla, J., & other authors. (2011). Unique Cell Adhesion and Invasion Properties of *Yersinia enterocolitica* O:3, the Most Frequent Cause of Human Yersiniosis. *PLoS Pathogens* **7**, e1002117.
- Young, G., Badger, J. L. & Miller, V. L. (2000). Motility is required to initiate host cell invasion by *Yersinia enterocolitica*. *Infection and Immunity* **68**, 4323-6.

Figure Legends:

Figure 1. *Y. enterocolitica* O:3 and O:8 mediated control of cell surface factors implicated in host cell interactions. Model of virulence factor expression of *Y. enterocolitica* O:3 and O:8 in response to temperature leading to the production of different *Yersinia* surface factors and distinct cell adhesion and invasion properties (Uliczka *et al.* 2011).

Figure 2. Interaction of RovA and H-NS with the *inv* promoter region of *Y. enterocolitica* O:3. The RovA activator protein binds to two independent binding sites between the IS1667 encoded promoter (P_{IS1667}) and the *inv* promoter (P_{inv}), and to one site upstream of the inserted IS1667 element. Binding of RovA to the two AT-rich binding sites between the promoters, of which one is encoded by IS1667, is required to alleviate binding of the silencing nucleoid-associated protein H-NS.

Fig. 1 Uliczka & Dersch 2011

Fig. 2 Uliczka & Dersch 2011