Journal of Life Science 2005, Vol. 15. No. 2. 202~210

Characterization of *Salmonella* Adhesins Required for Colonization of Animals

Young Hee Kim, Sam Woong Kim and Ho Young Kang*

Department of Microbiology, Pusan National University, 30 Jangjeon-dong, Geumjeong-gu, Busan 609-735, Korea

Received February 18, 2005 / Accepted March 18, 2005

Following ingestion, *Salmonella* must adhere to and colonize the intestinal epithelium of the host in order to establish infection. *S. typhimurium* synthesize several appendages that are believed to mediate attachment. These include type 1 fimbriae, plasmid-encoded (PE) fimbriae, long polar (LP) fimbriae, and thin aggregative fimbriae. However, the precise roles of these putative adhesins remain unclear, due to conflicting data in the literature. We constructed strains lacking four different fimbriae including type 1 fimbriae, PE fimbriae, LP fimbriae, and thin aggregative fimbriae, as well as strains lacking each fimbriae alone. In cell culture adhesion assays, these mutants adhered to several mammalian cell lines as well as wild-type *S. typhimurium*. These strains were also screened for virulence in mice, and all strains were virulent or nearly as virulent as their wild-type parents. In contrast, When a strain lacking four fimbriae was screened for virulence in chicks, it was found to be highly attenuated. This suggests a role for either type 1 fimbriae, PE fimbriae, LP fimbriae or thin aggregative fimbriae or a combination of thease fimbriae in the colonization of chicks. It also suggests that differences exist with respect to the surface structures that mediate attachment of *Salmonella* in chicks as compared with mice.

Key words – *Salmonella* fimbrial mutants

Salmonellosis represents one of the most widespread communicable bacterial diseases in the United States[25]. A report from the U. S. Centers for Disease Control and Prevention states that *Salmonella* accounted for 69% of the bacterial food borne disease outbreaks from 1998 to 1992[5]. The most common cause of *Salmonella* outbreaks is undercooked eggs, however, humans also commonly acquire *Salmonella* through ingestion of contaminated meats, dairy products, of water[5,15,32].

Following ingestion, *Salmonella enterica* serovar Typhimurium (*S. typimurium*) is capable of colonization of the intestinal tract of a wide variety of animal hosts[10]. The initial stages of infections, including adherence to and invasion of the intestinal mucosa, are essential for pathogenesis. Importantly, adherence and invasion are believed to be two separate events in *Salmonella* pathogenesis. This is based on the observation that invasion-defective mutants are fully capable of adhering to a variety of different cells lines[6,9, 11,13,17]. Much research has focused on the mechanism of invasion, and great strides have been made towards understanding this process[7]. In contrast, many fewer studies have focused on the process of adherence of *Salmonella* to intestinal epithelial cells, or to the M cells overlying the gut associated lymphoid tissue (GALT).

***Corresponding author**
Tel : +82-51-510-2266, Fax : +82-51-514-1778
E-mail : hoykang@pusan.ac.kr

Several groups have exmined the role of surface structures (including both flagella and fimbriae) in the virulence of *S. typhmurium*. Lockman and Curtiss showed that mutants lacking either flgella of type 1 fimbriae alone retained full virulence in mice, while mutants lacking both were significantly attenuated[22]. Consequently, it was proposed that both appendages mediate attachment to mouse cells, and if one adhesin is absent, the other could compensate. Independently, Van Der Velden *et. al.* also showed that mutants lacking type 1 fimbriae alone were fully virulent in mice[34], while Lee *et al.* demonstrated that mutants lacking the type 1 fimbriae were fully virulent in chicks[20].

In subsequent studies, Baumler and co-workers characterized the role of two additional fimbrial adhesins, the plasmid-encoded (PE) fimbriae[1] and the long polar (LP) fimbriae[2] in the virulence of *S. typhimurium*. Using an organ culture model, they showed that mutants unable to synthesize the PE fimbriae were associated with the murine small intestine in lower numbers than a wild-type strain[1]. These mutants also displayed a two fold increase in the oral LD_{50} in mice[1]. Similarly, studies using a strain unable to synthesize the LP fimbriae showed that this mutant also adhered less well to the murine small intestine than wild type[2]. However, the *lpf* mutant specifically appeared less adherent to cells within Peyer's patches, but not the villous intestinal tissue. In their studies, the *lpf* mutants also showed reduced virulence in mice, displaying

a five-fold higher LD_{50} than wild type[2].

The Agf fibers whice are known to bind soluble fibronectin, are homologous to the curli organelles of *E. coli*[33]. Sukupolvi *et. al.* showed that a strain containing a knockout mutation in the *agf*A gene adhered 50-fold less well than wild type to a cell line derived from mouse intestinal epithelium[33]. In contrast, Van Der Velden *et. al.* demonstrated that a mutant lacking thin aggregative fimbriae was only slightly less virulent than wild type[34].

As discussed above, most studies examining the role of fimbriae have shown that mutations eliminating their synthesis have only small effects on virulence in an animal model. It has been surmised that multiple fimbriae (or other surface appendages) function as adhesins, and that if one is absent due to mutation, another may simply substitute[22,34]. If this is true, the individual contribution of each adhesin may be difficult to evaluate. Consequently, it was our goal to construct strains lacking multiple fimbriae and to evaluate the ability of these strains to adhere to cells in culture, and to cause disease in different animal models. Although a similar study has been conducted previously, the current study contains more (and in some cases conflicting) data.

Specifically, we constructed afimbriate mutants in several virulent *S. typhimurium* backgrounds, and examined the ability of these strains to adhere to several mammalian cell lines in vitro. We also assessed the effect of the quadruple mutant on virulence in mice and day-of-hatch chicks. Our results indicate that *S. typhimurium* may use different surface structures to adhere to mouse cells as compared with chicken cells. Importantly, this study demonstrates the need to examine the effect of mutations in multiple animal models and perhaps, in multiple sub-strains. It also cautions us to avoid generalizing results from *Salmonella* virulence studies conducted solely in mice.

Materials and Methods

Media, bacterial strains, plasmids and phenotypic screens

Standard media were used for cultivation of bacteria including Lennox broth[21], Luria Bertani broth[23], CFA agar[12] and *EBU* agar[4]. Difco agar was added to Lennox broth at 1.5% for base agar (L agar). CFA agar was supplemented with 0.01% Congo red dye when screenig for *agf* mutations. Antibiotics (Sigma, St. Louis, MO) were added to media at the following concentrations: tetracycline (Tet), 15 μg/ml; kanamycin (Kan), 50 μg/ml; spectionomycin (Spc), 200 μg/ml; streptomycin (Str), 50 μg/ml; ampicillin (Amp), 100 μg/ml; and chloramphenicol (Cat), 25 μg/ml. All strains and plasmids used in this study are listed in Table 1. *S. typhimurium* strains were constructed that lack type 1 fimbriae (Fim), long polar fimbriae (Lpf), plasmid-encoded fimbriae (Pef) and thin aggregative fimbriae (Agf). This was done by transducing mutations in the individual fimbriae genes into the virulent *S. typhimurium* strains UK-1, SL1344, SR-11 and ATCC14028 by P22 HT*int* transduction[31] or ES18 transduction[19]. Transductants were selected based on the antibiotic resistance markers associated with each mutation. The mutations were also sequentially transduced into the wild-type strain backgrounds in order to create mutants lacking all four fimbriae (Table 1). The source of the *pefC*::*tet* and Δ(*lpfABCDE*)::*kan* mutations were strains AJB7 and AJB103, respectively, and these were kindly provided by A. Baumler. Transductants containing the *fimA* mutation were identified based on their inability to agglutinate yeast cells in the absence of mannose, as described previously [18]. The *agfA*::*spc/str* mutation was constructed by inserting the Ω fragment, encoding resistance to spectinomycin and streptomycin, into the *agfA* gene of pHUB100. Specifically, the plasmid pUC4::Ω was digested with SmaI, and a 2 kb fragment containing the omega fragment was gel purified and ligated with XmnI-digested pHUB100[33] (containing the *agfBAC* operon). The resultant plasmid, pCJN2, was verified by restriction analysis. pCJN2 was then digested with SalI and the 4.1 kb fragment (containing the *agfA*::Ω insertion mutation) was cloned into the *Sal*I site of the Kan-resistant suicide vector pJP5063[28]. The resulting plasmid, pCJN4, was transformed into MGN-617(λ *pir* Δ*asd*), and subsequently conjugated into χ3761 (UK-1 wild type). Exconjugates were selected on an LB plate containing spectinomycin (but lacking diaminopimelic acid). Several recombinants which resulted from double cross- over events were obtained. One such strain, χ8341, contained the *agfA*::*spc/str* mutation on the bacterial chromosome as evidenced by the lack of congo red binding and Kan sensitivity (indicating loss of the vector pJP5063).

Molecular techniques used to verify the presence of the fimbrial mutations in the mutant strains.

Either PCR or southern hybridization was used to verify the presence of the fimbrial mutations in all strains described

Table 1. Bacterial strains and plasmids

Strain or plasmid	Genotype	Reference or source
E.coli strains		
MGN-617	*λpir/thi-1 thr-1 leuB6 glnV44 tonA21 lacY1 recA* RP4-2-Tc::Mu *ΔasdA4* Δ(*zhf-2*::Tn*10*)	Megan Health
Salmonella typhimurium strains		
UK-1 derivatives		
χ3761	wild type	8
χ8252	Δ(*lpfABCD*)::*kan*	this study
χ8259	*fimA*::*cat*	this study
χ8273	*pefC*::*tet*	this study
χ8308	*agfA*::*spc/str*	this study
χ8307	Δ(*lpfABCD*)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	this study
χ8341	*agfA*::*spc/str*	this study
SL1344 derivatives		
χ3339[a]	*hisG*	16
χ4439	*hisG fimA*::*cat*	24
MWR1523	*hisG* Δ(*lpfABCD*)::*kan*	this study
MWR1526	*hisG fimA*::*cat*	this study
MWR1541	*hisG agfA*::*spc/str*	this study
MWR1544	*hisG pefC*::*tet*	this study
MWR1550	*hisG* Δ(*lpfABCD*)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	this study
SR11 derivatives		1
AJB7	*pefC*::*tet*	A. Baumler
AJB103	Δ(*lpfABCD*)::*kan*	22
χ3181	wild type	this study
MWR 1521	Δ(*lpfABCD*)::*kan*	this study
MWR 1524	*fimA*::*cat*	this study
MWR 1539	*agfA*::*spc/str*	this study
MWR 1542	*pefC*::*tet*	this study
MWR 1548	Δ(*lpfABCD*)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	this study
ATCC14028 derivatives		
14028(MWR398)	wild type	ATCC
MWR 1522	Δ(*lpfABCD*)::*kan*	this study
MWR 1525	*fimA*::*cat*	this study
MWR 1540	*agfA*::*spc/str*	this study
MWR 1543	*pefC*::*tet*	this study
MWR 1549	Δ(*lpfABCD*)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	this study
Plasmids		
pCJN2	pHUB100 with *agfA*::*spc/str*	this study
pCJN4	pJP5063 with *agfA*::*spc/str*	this study
pHUB100	*agfBAC*	34
pJP5063	oriR6K *mob* RP4	29
pUC4::Ω	pUC4 containing the Ω fragment encoding Spc^R and Str^R	B. Goldman

[a]Strain χ3339 is a mouse-passaged derivative of SL1344, the latter of which is cited and was obtained from B. A. D. Stocker.

in this study (both single and quadruple mutants). The primers 5′-ATTCATCACCCTGGCTATGGTTAC-3′ and 5′CGGGCGACATCAAAACAAGC-3′ were used in PCR experiments to verify the presence or absence of a *fimA* mutation. These primers amplify a 1 kbp fragment in the wild-type strains, or a 2.5 kbp fragment in the *fimA* mutant strains. To verify the *pefC* mutation, the primers 5′-CGGGGGCTGTAAACGCTAAC-3′ and 5′-CAGGGTTCCAGTGGTGTTTC TTG-3′ were

used to amplify a 4 kbp fragment in the wild type strains, or a 5.5 kbp fragment in the *pefC* mutant strains.

Tissue culture adherence assays

In order to examine the ability of the *S. typhimurium* fimbrial mutants to adhere to cells in culture, we used three different cell culture lines, including Int-407[14], HEp-2[26] and J774[29]. The adherence assay was modified from a method developed by Galan and Curtiss[11]. Briefly, a cell line was seeded onto 24-well tissue culture plates and grown in Minimal Essential Medium medium (MEM; GibcoBRL, Grand Island, NY) supplemented with 10% fecal calf serum (FCS; Hyclone, Logan, UT), and 2 mM glutamine until 70% to 90% confluency. The cell culture medium was replaced with fresh medium one day, and subsequently, one hour prior to the assay. Log-phase, *S. typhimurium* cultures (OD at 600 nm of 0.2 to 0.4) growth in Luria Bertani broth were added directly to the monolayers in triplicate at a multiplicity of infection (MOI) of approximately one. Plates were incubated at 37℃, 5% CO_2 and bacteria were allowed to adhere to host cells for 30 minutes. During the incubation period, the bacterial cultures were diluted and plated on L agar to determine the exact number of bacteria in the inoculum. Following the 30 minute incubation, the cell culture medium was removed, and the monolayers were washed twice with 1 ml buffered saline containing gelatin (BSG; 0.85% NaCl, 0.01% gelatin, 2.2 mM KH_2PO_4 and 4.2 mM Na_2HPO_4). After the second wash, 0.5 ml BSG containing 0.1% sodium deoxycholate was added to each well to detach and lyse the host cells. The titer of bacteria in each well was determined by diluting cells in BSG and plating serial dilutions on L agar. The percent adherence of each strain is calculated as the number of cells adhered/ number of cells added to the well.

Animal studies.

LD_{50} values for the *S. typhimurium* strains were determined following peroral inoculation of BALB/c mice. Six to eight week-old female BALB/c mice were used for all studies. Mice were obtained from Harlan Spargue Dawley (Indianapolis, IN) and acclimated at least one week prior to inoculation. Bacteria for inculation were grown overnight in Luria Bertani broth from a frozen stock, then diluted 1:200 in fresh Luria broth the following morning. Cultures were aerated at 37℃ for approximately three hours until an optical density at 600 nm of 0.8 to 1.0 was reached. The cells were then pelleted, resuspended in BSG, and dilutions were made in BSG to obtain the appropriate doses. To determine bacterial titers, dilutions in BSG were made as appropriate and cells plated on L agar. Mice were deprived of food and water for four to six hours prior to infection. Peroral inoculations were made using a micropipet tip placed immediately behind the incisors and by ejecting 20 μl of *Salmonella* suspension. Food and water were returned 30 minutes post-inoculation. Mice were observed for a four week period following inoculation. LD_{50} determinations were made using the method of Reed and Meunch[30] with four mice per inoculum dose. LD_{50} values for *S. typhimurium* strains were also determined following peroral inoculation of white leghorn chicks. Fertile white leghorn chicken eggs were purchased from Spafas (Roanoke, IL) and incubated 21 days in a hatcher-incubator. After hatching, chicks were placed in Nalgene cages and no food or water was given prior to infection. Chicks were infected within 8 hours of hatch with either wild-type UK-1 or the afimbriate mutant χ8307. The bacterial inocula were prepared as described above for mice. Peroral inoculations were made using a micropipet tip placed in the beak and by injecting 100 μl of *Salmonella* suspended in BSG. Food was supplied 30 minutes post-inoculation. For each LD_{50} experiment, chicks were observed for a two week period. LD_{50} determinations were made using the method of Reed and Muench[31] with five chicks per inoculum dose.

Results

Adherence of *S. typhimurium* strains lacking single or multiple fimbriae to cells in culture

Salmonella produce multiple fimbriae, and it has been suggested that they function synergistically to mediate attachment to host cells. Studies that have evaluated the individual role of the different fimbriae have illustrated that mutations eliminating their synthesis have only small effects on virulence in an animal model. Consequently, we decided to construct a strain lacking multiple fimbriae, and to determine if this strain is less adhesive and less virulent in different animal models. Sets of isogenic strains were constructed that contain mutations in fimbrial genes (either singly or in combination) in several different virulent *S. typhimurium* strain backgrounds (Table 1). We considered that mutants lacking fimbriae (either singly or in combination) may adhere less well to host cells than a wild-type

parent strain. Therfore, we examined the capacity of the *fimA*, *pefC*, Δ(*lpfABCDE*) and *agfA* mutants, as well as the mutants lacking all four fimbriae, to adhere to Int-407, HEp-2 and J774 cell lines. The parent strains adhered to Int-407 cells at 10.4% (UK-1), 9.6% (SL1344), 12.9% (SR-11) and 3.9% (ATCC14028). None of the individual mutations significantly reduced the ability of the parent strain to adhere to Int-407 cells (Table 2). The data obtained for the *lpf*, *fim* and *pef* mutants are consistent with other studies in which similar mutations had no effect on adherence of *S. typhimurium* to Int-407 cells[3]. Interestingly, in each strain background, strains lacking all four fimbriae also adhered as well as their wild-type parents, suggesting that none of these fimbriae mediate attachment to Int-407 cells. We measured adherence of the UK-1 derivatives to HEp-2 and J774 cells as illustrated in Table 3. The wild-type UK-1 parent adhered at 11% to HEp-2 cells and 19.8% to J774 cells. Again, strains containing fimbrial mutations (either singly or in combination) adhered as well as their wild-type parents to these cell lines. Consequently, we conclude that none of these fimbriae appear to mediate attachment to HEp-2 or J774 cells. This is inconsistent with previous data in which a mutation in *lpfC* resulted in a 10-fold reduction in adherence to HEp-2 cells[3]. Possible explanations for this discrepancy are discussed below.

Virulence of *S. typhimurium* strains lacking fimbriae in mice

To assess the role of the various fimbriae in mouse vurulence, LD_{50} studies were conducted as described above. As shown in Table 4, strains lacking all four fimbriae were as virulent as their UK-1 and SL1344 parent strains. In the SR-11 strain background, the LD_{50} of the afimbriate mutant was only 3.5-fold higher than its wild-type parent. This data is inconsistent with that reported previously[34] in which an SR-11 afimbriate mutant displayed an LD_{50} that was 26-fold higher than the parent. Possible explanations for this descrepancy are discussed below. In addition, the quadruple fimbrial mutant was only somewhat attenuated (13-fold) in the ATCC14028 bacground. Taken together, these data suggest that none of the four fimbriae described in this study play a significant role in the virulence of orally

Table 2. Relative level of adherence of several fimbrial mutants to Int-407 cells[1]

Relevant genotype	Strain background			
	UK-1	SL1344	SR-11	ATCC14028
Wild type	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
Δ(*lpfABCD*)::*kan*	1.7±1.1	0.8±0.1	0.7±0 .02	1.6±0.4
fimA::*cat*	2.3±1.2	1.7±0.4	1.2±0.3	1.7±0.1
pefC::*tet*	0.8±0.2	1.2±0.3	1.2±0.3	1.8±0.2
agfA::*spc/str*	1.5±1.1	0.8±0.1	1.1±0.4	1.4±0.1
Δ(*lpfABCD*)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	0.9±0.1	1.1±0.3	1.0±0.2	2.1±1.0

[1]Values given are normalized and expressed as a fraction of each wild-type control±the standard error. The data represent an average of two trials with strains assayed in triplicate in each trial. Actual strains assayed include UK-1 derivatives: χ3761, χ8252, χ8259, χ8273, χ8308, χ8307; SL1344 derivatives: χ3339, MWR1523, MWR1526, MWR1544, MWR1541, MWR1550; SR-11 derivatives: χ3181, MWR1521, MWR1524, MWR1542, MWR1539, MWR1548; ATCC14028 derivatives: MWR398, MWR1522, MWR1525, MWR1543, MWR1540, MWR1549

Table 3. Relative level of adherence of several fimbrial mutants to Hep-2 and J774 cells[1]

Relevant genotype	Cell line	
	HEp-2	J774
Wild type	1.0±0.0	1.0±0.0
Δ(*lpfABCD*)::*kan*	1.9±0.6	1.1±0.2
fimA::*cat*	2.4±0.5	1.4±0.1
pefC::*tet*	2.3±0.4	1.0±0.1
agfA::*spc/str*	1.7±0.2	1.5±0.4
Δ(*lpfABCD*)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	2.2±0.3	1.4±0.0

[1]Values given are normalized and expressed as a fraction of the wild-type control±the standard error. The data represent an average of two trials with strains assayed in triplicate in each trial. Actual strains assayed include UK-1 derivatives: χ3761, χ8252, χ8259, χ8273, χ8308, χ8307.

administered *S. typhimurium* strains UK-1, SL1344 and SR-11 in mice. Furthermore, one or more of these fimbriae may function in the pathogenesis of *S. typhimurium* ATCC14028 in mice, however, clearly all strains possess adhesins other than these fimbriae which are essential for mouse virulence.

Virulence of *S. typhimurium* strains lacking in chicks.

LD_{50} studies were also conducted with day-of-hatch chicks in order to investigate the role of the various fimbriae in this animal. The results of two independent experiments are shown in Table 5. Our data indicate that χ8307 (the afimbriate mutant in the UK-1 background) is highly attenuated. This suggests a role for either type 1 fimbriae, PE fimbriae, LP fimbriae, and thin aggregative fimbriae or a combination of these fimbriae in the colonization of chicks.

Discussion

Mutants were constructed lacking type 1 fimbriae, plasmid-encoded fimbriae, long polar fimbriae, and thin aggregative fimbriae, both singly and in combination in several different *S. typhimurium* backgrounds. All mutants lacking fimbriae adhered to as well as the wild-type parents to Int-407, HEp-2 and J774 cells in culture. Given the observation that strains lacking all four fimbriae are either fully virulent in mice or only slightly attenuated, it is perhaps not surprising that they adhered well to these mammalian cell lines. Baumler *et.al.*[3] and Van der Velden *et.al.*[34] published similar studies previously, however, the data described herein differ from their reports in several respects. First, our data show no effect of an *lpf* mutation on adherence to HEp-2 cells. This experiment was repeated in four different strain backgrounds as described above, which adds strength to our conclusion that long polar fimbriae do not mediate attachment to the HEp-2 cell line. Second, Van der Velden *et.al.* conclude that the simultaneous inactivation of genes involved in the biosynthesis of the four distinct fimbriae markedly attenuates *S. typhimurium* in mice[34]. This conclusion is based on data obtained using the SR-11 strain background, in which the quadruple mutant exhibited an LD_{50} more than 26-fold higher than the parent strain. Our data clearly do not support this conclusion. Instead, we show little or no effect on virulence in mice when the four fimbriae are eliminated by mutation in the UK-1, SL1344, and SR-11 strain backgrounds. Furthermore, the LD_{50} of an ATCC14028 derivative lacking the four fimbriae was only 13-fold higher that the wild type parent. Our study also differs from that of Van der Velden *et.al.*, in that we assessed the virulence of a strain lacking all four fimbriae in day-of-hatch chicks while they did not use other animal models. Surprisingly, our strain lacking the four fimbriae was significantly less virulent in chicks than wild-type UK-1.

It is a concern that different studies report strikingly dissimilar results when examining the same biological

Table 4. Effect of multiple fimbrial mutations on peroral LD_{50s} in mice

Relevant genotype	LD_{50} In strain background[1]			
	UK-1	SL1344	SR-11	ATCC14028
Wild type	3.0×10^5	2.6×10^5	1.1×10^5	4.9×10^5
Δ(*lpf*ABCD)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	3.1×10^5	3.0×10^5	3.9×10^5	6.5×10^6

LD_{50} values are given for the wild type strain in each background and the mutant lacking all four fimbriae. Actual strains used in mouse infections include: UK-1 derivatives: χ3761, χ8307; SL1344 derivatives: χ3339, MWR1550; SR-11 derivatives: χ3181, MWR1548: ATCC14028 derivatives: MWR398, MWR1549.

Table 5. Effect of multiple fimbrial mutations on virulence in chicks infected perorally

		Experiment 1		Experiment 2	
			# chicks		# chicks
Strain	Relevant genotype	Inoculum dose	dead/total	Inoculum dose	dead/total
χ3761	Wild-type UK-1	2.6×10^3	5/5	2.6×10^4	5/5
		2.6×10^2	4/5	2.6×10^3	4/5
		2.6×10^1	3/5	2.6×10^2	1/5
χ8307	Δ(*lpfABCD*)::*kan fimA*::*cat pefC*::*tet agfA*::*spc/str*	2.5×10^3	2/5	3.2×10^4	1/5
		2.5×10^2	1/5	3.2×10^3	0/5
		2.5×10^1	1/5	3.2×10^2	1/5

phenomena. A possible explanation for these inconsistencies is simply that subtle differences exist in the way adherence and/or virulence is measured, and these differences may affect the outcome of the experiment. For example, in the report in which Baumler *et.al.* examined the contribution of fimbriae to attachment to various cell lines, the authors used HEp-2 cells that were fixed with gluteraldehyde or, separately, they performed the adherence assys at 4℃[3]. Also, Baumler *et.al.* used a strain that had an insertion in *lpfC*, whereas we used a strain that contained a deletion of the entire *lpf* operon. Furthermor, in the study by Van der Velden *et.al.*[34], the authors used a very different means to measure oral vurulence in mice as compared with the method described here. In the former studies, mice were infected intragastrically with overnight *S. typhmrium* cultures. Also, in that study, there is no mention of fasting mice prior to infection. In our report, we performed peroral infections of fasting mice, using log-phase *Salmonella* cells for the inocula. We postulate that our growth conditions may optimize infection because (i) fasted mice have a higher gastric pH, and thus provide a greater uniformity of infection as fewer bacterial cells die and (ii) log phase cells display enhanced expression of SPI-1 *inv* genes, permitting bacteria to invade without having to first adapt to the intestinal microenvironment. Another difference between our study and that of Van der Velden *et.al.*, is that different mutations in the *fim, agf* and *lpf* operons were used. In pointing out these differences, our goal is not to convince readers that one study is more accurate than the other. Instead, we hope to caution readers that the protocols one chooses to use in the laboratory to measure various attributes of virulence, may produce different results, depending on the exact methodologies used. Also, it is also important to note that the different *S. typhimurium* strain backgrounds may be genetically different, consequently, it may be prudent to study virulence using more than one strain background.

Our data clearly suggest that *S. typhimurium* must synthesize additional surface structures to mediate attachment to mouse intestinal epithelial cells. Van der Velden *et.al* concluded this as well, and even demonstrated fimbrial-like structures on cells that hardbored mutations in *fim, lpf, agf, and pef*[34]. McClelland *et.al.*[24] published the genome sequence of *S. typhimurium*, and in their report, the authors identified a total of 13 putative fimbrial operons, including: *agf, lpf, fim, pef, bcf, saf, stb, stc, std, stf, sth, sti and stj*. One of these, *agf*, represents fimbriae belonging to the nucleator-dependent assembly type, while the remaining 12 are of the chaperone-usher assembly class[24]. Interestingly, two of these (*sti* and *stj*) appear to be absent from the *S. typhi* genome [24]. One of these is likely to be identical to the *Stf* fimbriae indentified in R. curtiss III's lab[27].

Strain χ8307, which harbors mutations in the *fim, lpf, agf,* and *pef* genes, is fully virulent in mice, yet this strain appears significantly attenuated in chicks. This suggests a role for either type 1 fimbriae, PE fimbriae, LP fimbriae, or thin aggregative fimbriae (or a combination of these fimbriae) in the colonization of chicks. It also leads us to conclude that differences exit with respect to the surface structures that mediate attachment of *S. typhimurium* in chicks as compared with mice. Also, it suggests that it may be prudent to test *S. typhimurium* virulence in more than one animal model, or at least to consider the relevance of the various animal models that are used. Specifically, mice are generally used as a model for typhoid fever, chicks are often used to study persistence and shedding of *Salmonella,* and calves are used as a model for grastroenteritis. To our knowledge, there is only one other example where contrasting results were obtained when using different host species in *S. typhimurium* experimental infections. In this example, Lee *et. al.* showed that a Fla$^-$ Mot$^-$ *S. typhimurium* strain is attenuated in one-day old chicks, with an LD_{50} more than three log units higher than the parent strain[20]. In contrast, the indentical Fla$^-$ Mot$^-$ *S. typhimurium* mutant was shown to be fully virulent in mice[22].

Acknowledgements

This work was supported by Korea Research Foundation Grant (KRF-2003-003-E00036).

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초록 : 동물세포의 부착에 관여하는 살모넬라 유전자의 특성 연구

김영희 · 김상웅 · 강호영*

(부산대학교 자연과학대학 미생물학과)

살모넬라는 질병을 일으키기 위해서 장내의 상피세포에 먼저 부착하여 집락을 이루게 된다. 이에 따라 살모넬라는 부착에 관여하는 세포내 몇몇 소기관들을 합성한다. 이러한 소기관에는 type 1 fimbriae, plasmid- encoded fimbriae, long polar fimbriae 그리고 thin aggregative fimbriae 등이 있다. 본 논문에서는 type 1 fimbriae, thin aggregative fimbriae, LP fimbriae, 그리고 PE fimbriae 각각을 결손시킨 변이주와 4종류를 모두 결손시킨 변이주를 만들 수 있었다. 변이주들에 대해서 세포배양 부착성 실험을 한 결과, 각각을 결손시킨 변이주들은 몇몇 mammalian 세포주에서 야생형 살모넬라와 동일한 양상으로 부착성을 나타내었고 마우스 실험에서 야생형과 거의 마찬가지로 독성을 가지고 있었다. 반면, 4 종류의 fimbriae가 모두 결손된 균주는 닭에서는 독성을 나타내었으나 마우스에서는 매우 약독화되어있음을 확인할 수 있었다. 이상의 결과로부터 오는 차이점은 닭과 비교하여 마우스에서 살모넬라의 부착을 매개하는 표면 구조에 연관성이 있음을 제시하였다.