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Contribution of Fimbrial Operons to Attachment to and Invasion of Epithelial Cell Lines by *Salmonella typhimurium*

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The role of the *Salmonella typhimurium* fimbrial operons, *lpf*, *fim*, and *pef*, in adhesion to and invasion of epithelial cell lines was investigated. An *S. typhimurium lpfC* mutant was unable to adhere to or to invade HEp-2 cells, while an *S. typhimurium fim* deletion mutant did not attach to or enter HeLa cells. These results suggest that adhesion is a prerequisite for invasion and that distinct fimbrial adhesins select different target cells for invasion by *S. typhimurium*.

Salmonella typhimurium causes diseases in a wide variety of animals, ranging from enteric fever in mice to gastroenteritis in humans. At the onset of these infections, *S. typhimurium* attaches to and penetrates the intestinal mucosa of its mammalian host. Interestingly, *S. typhimurium* preferentially invades epithelial cells located within ileal Peyer's patches or at the tip of villi in the ileum (7, 8, 20, 23, 27). Despite the progress made on elucidating the mechanisms of invasion (4, 6, 16, 19, 21, 22, 26, 28, 36), it is still not known which factors determine the epithelial cell lineage invaded by *S. typhimurium* in the intestine. For instance, why is it that in the follicle-associated epithelium of Peyer's patches, *S. typhimurium* invades M cells but not enterocytes (8, 27)? There is evidence that fimbriae may actually target *S. typhimurium* to a particular cell type in the intestine. For example, fimbriae have been shown to contribute to the tissue tropism of *S. typhimurium* for the murine villous small intestine (2) and murine Peyer's patches (3). These results prompted us to investigate the role of fimbrial adhesins in selecting a target cell type for *S. typhimurium* invasion.

Previous studies of the role of fimbrial adhesins of *S. typhimurium* in attachment to epithelial cells have used cultures enriched for fimbriated or nonfimbriated cells (14, 25, 34) or strains not stably nonfimbriated (13). Considering the lack of information about factors which influence fimbrial phase variation in vivo and variability in the fimbriation of cells carrying intact fimbrial operons, these reports may be difficult to evaluate. We therefore used a genetic approach to investigate the role of the three known *S. typhimurium* fimbrial operons, *fim* (10, 11, 37, 40), *lpf* (1), and *pef* (18), which encode type 1 fimbriae, long polar fimbriae, and plasmid-encoded fimbriae, respectively, in interaction with epithelial cells. Aerobic static broth cultures of *S. typhimurium* (adjusted to about 5×10^8 bacteria per ml) were used as inocula for the infection of tissue culture cells throughout this study since this growth condition enriches for fimbriated bacteria (12). Cell lines were grown on 24-well plates to about 70% confluency and fixed with glutaraldehyde for adhesion assays, as described previously (32), or used directly for gentamicin protection (invasion) assays, as described by Lee and coworkers (33). Three wells were infected with 0.05, 0.1, and 0.15 ml of bacterial culture, respectively, to correct for variations in the inoculum. By this method, we consistently infected one well with an inoculum of about 5×10^7 bacteria. All experiments were repeated at least four times independently. As an additional control, adhesion experiments were also performed at 4°C with live cells to ensure that glutaraldehyde fixation did not affect the numbers of adherent bacteria recovered (data not shown). Student's *t* test (small-sample inference concerning the difference between two means) was used to evaluate the significance of the differences in adhesion or invasion observed for different strains. The probability (*P*) that the difference between two strains was significant is given below.

The adhesion of *S. typhimurium* to HEp-2 cells has been proposed to be mediated by type 1 fimbriae (42). However, to date, genetically defined *S. typhimurium fim* mutants have not been tested for adhesion to HEp-2 cells. Lockman and Curtiss have described the construction of an *S. typhimurium fim* deletion mutant (35). We found no significant effect ($P > 0.1$) of this deletion on the ability of *S. typhimurium* to adhere to HEp-2 cells (Fig. 1A). In contrast, a mutation in *lpfC*, encoding the putative fimbrial usher of the *lpf* operon (1), resulted in 10-fold-reduced adhesion of *S. typhimurium* to HEp-2 cells ($P < 0.005$) (Fig. 1A). Adhesion of the *S. typhimurium lpfC* mutant (AJB1) to HEp-2 cells could be restored by the introduction of a plasmid (pMS1054) (1) carrying an intact *lpfC* allele (Fig. 1A). Furthermore, a mutation in *pefC*, the gene encoding the putative usher of plasmid-encoded fimbriae, did not affect adhesion to HEp-2 cells (data not shown). Similarly, mutations in *lpfC* and *pefC* or deletion of *fim* had no effect on the adhesion of *S. typhimurium* to MDCK ($P > 0.1$; Fig. 1C), T-84, or Int-407 cells (data not shown). Generally, the percentage of the *S. typhimurium* inoculum which was cell associated was similar to that described by Francis and coworkers for a similar experimental setup (17). In conclusion, these data show that the *lpf* fimbrial operon is necessary for the adhesion of *S. typhimurium* to HEp-2 cells.

An *S. typhimurium fim* deletion mutant (AJB4) adhered to HeLa cells in numbers sevenfold lower than those of the isogenic wild-type strain (AJB3) ($P < 0.005$; Fig. 1E). Adhesion of the *S. typhimurium fim* deletion mutant (AJB4) to HeLa cells could be restored by the introduction of a plasmid (pISF101) carrying the entire *S. typhimurium fim* operon (9). Furthermore, *S. typhimurium* strains carrying mutations in *lpfC*

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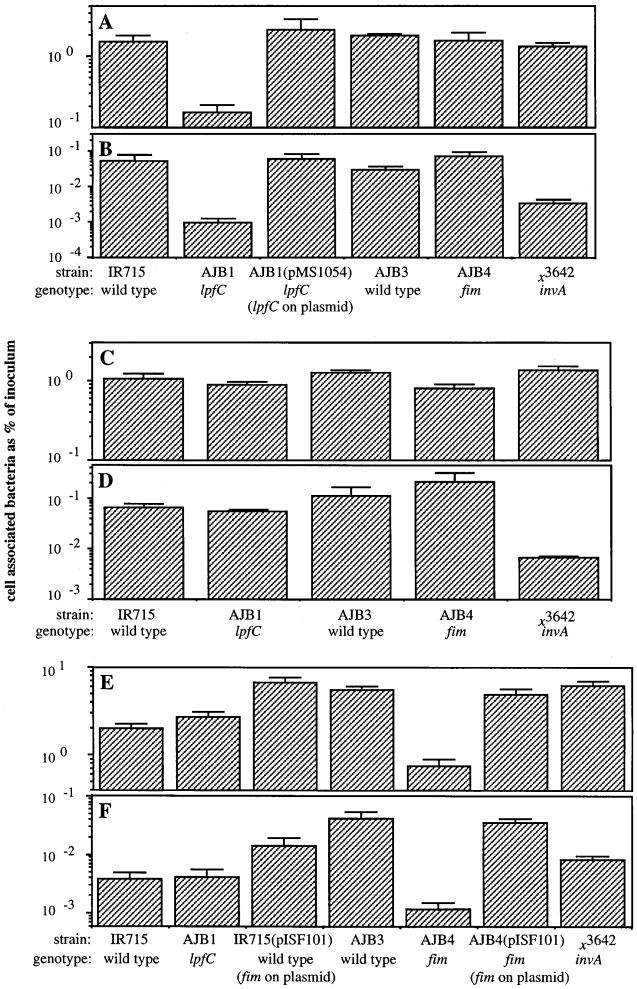

FIG. 1. Adhesion to (A, C, and E) and invasion of (B, D, and F) HEp-2 (A and B), MDCK (C and D), and HeLa (E and F) cells by strains of *S. typhimurium*. Data are CFU expressed in mean percentages of the inoculum ± standard errors. Strain designations and relevant genotypes of strains are indicated below bars. AJB3 and AJB4 are nalidixic acid-resistant derivatives of strains $_x$4252 and $_x$4253, respectively (35).

(AJB1; Fig. 1E) and *pefC* (AJB7; data not shown) adhered to HeLa cells as well as did their isogenic parent (IR715) (38). These data clearly show that the *fim* operon is involved in the adhesion of *S. typhimurium* to HeLa cells and therefore confirm a previous report by Tavendale and coworkers (42). Interestingly, we observed that *S. typhimurium* strains derived from isolate SR-11 adhered to HeLa cells in numbers higher than those of strains derived from isolate ATCC 14028 ($P < 0.005$). Introduction of the *fim* operon on a plasmid (pISF101) increased adhesion to HeLa cells by IR715 to the levels of SR-11 derivatives ($P < 0.005$). Mannose-sensitive agglutination mediated by type 1 fimbriae has been shown to be a function of both bacterial numbers and the length of time bacteria and yeast cells are in contact (12). To determine whether the differences in adhesion between SR-11 and ATCC 14028 are reflected in differences in the expression or adhesiveness of type 1 fimbriae, the minimal number of bacteria able to mediate mannose-sensitive agglutination of yeast cells after a 2-min incubation was determined (12, 40). IR715, a derivative of ATCC 14028, showed a fivefold increase in the bacterial CFU (2×10^7) required to mediate mannose-sensitive yeast agglutination compared with that of the SR-11 derivative AJB3 (4×10^6). In addition, upon introduction of a plasmid encoding the *S. typhimurium fim* operon, yeast agglutination of strain IR715 was increased (4×10^5). Yeast agglutination was not observed with the *S. typhimurium fim* deletion mutant (AJB4). However, introduction of the *S. typhimurium fim* operon on plasmid pISF101 into the *S. typhimurium fim* deletion mutant (AJB4) restored yeast agglutination (7×10^5). Thus, the abilities of *S. typhimurium* strains to mediate mannose-sensitive yeast agglutination corresponded well to the observed levels of adhesion to HeLa cells and were therefore consistent with a role for type 1 fimbriae in adhesion to HeLa cells. These data further indicated that *S. typhimurium* ATCC 14028 and SR-11 differ in the adhesiveness or level of expression of their type 1 fimbriae.

Our data show that distinct fimbrial operons (e.g., *lpf* and *fim*) mediate the adhesion of *S. typhimurium* to different epithelial cell lines (e.g., HEp-2 and HeLa cells). Obviously, fimbrial adhesins encoded by *lpf* and *fim* bind receptors which are expressed only on the surfaces of HEp-2 and HeLa cells, respectively. Similarly, in the mammalian intestine, fimbrial adhesins may recognize and bind surface epitopes which are present only on the apical membranes of their target cell types. Consequently, bacterial binding would be restricted to a set of cell populations carrying this surface receptor (Table 1). Therefore, our results are consistent with a role for fimbrial adhesins in targeting *S. typhimurium* to a particular cell lineage in the intestine. Indeed, in an intestinal-organ culture model, the *pef* fimbrial operon mediates attachment to the murine villous small intestine, whereas selective adhesion of *S. typhimurium* to murine ileal Peyer's patches is mediated by the *lpf* fimbrial operon (2, 3). In summary, these results support the hypothesis that during colonization of the intestine by *S. typhimurium*, the bacterial repertoire of fimbrial adhesins determines the epithelial cell types to which bacteria are able to attach (Table 1).

TABLE 1. Role of *S. typhimurium* fimbrial operons in adhesion to epithelial cells

Fimbrial operon	Adhesion in epithelial cell line or tissue (organism)[a]							
	HEp-2 cells (human)	T-84 cells (human)	Int-407 cells (human)	HeLa cells (human)	MDCK cells (dog)	Villous intestine (rat)[b]	Villous intestine (mouse)[c]	Peyer's patch (mouse)[d]
fim	−	−	−	+	−	+	ND	ND
lpf	+	−	−	−	−	ND	−	+
pef	−	−	−	−	−	ND	+	−

[a] ND, no data; +, mediates adhesion; −, no role in adhesion.
[b] Data are from reference 35.
[c] Data are from reference 2.
[d] Data are from reference 3.

It has been proposed previously that in *S. typhimurium*, adhesion mediated by fimbriae is necessary for invasion of cultured epithelial cell lines (14, 24, 29, 30). However, genetic evidence for the involvement of a particular fimbrial operon in invasion is lacking. We therefore tested *S. typhimurium* strains carrying mutations in the *lpf* (AJB1), *fim* (AJB4), and *pef* (AJB7) fimbrial operons for the ability to invade Int-407 (data not shown), T-84 (data not shown), HEp-2 (Fig. 1B), MDCK (Fig. 1D), and HeLa (Fig. 1F) cells. As a control, an *S. typhimurium* strain ($_{x}$3642) carrying a mutation in *invA*, a gene necessary for cell invasion, along with the *S. typhimurium* wild type, was tested in each experiment (19). All strains tested, except the noninvasive *S. typhimurium invA* mutant ($_{x}$3642) ($P < 0.05$), entered Int-407, T-84, and MDCK cells equally well (Fig. 1D). In contrast, two *S. typhimurium* strains, the *invA* mutant ($_{x}$3642; $P < 0.025$) and the *lpfC* mutant (AJB1; $P < 0.005$), showed defects in the invasion of HEp-2 cells (Fig. 1B). The 50-fold-reduced invasion rate of the *lpfC* mutant (AJB1) could be restored to wild-type levels by the introduction of an intact *lpfC* allele on a plasmid (pMS1054). These results show that the *lpf* operon is necessary for invasion of HEp-2 cells. In HeLa cells, the *S. typhimurium fim* deletion mutant (AJB4; $P < 0.005$) and the *S. typhimurium invA* mutant ($_{x}$3642; $P < 0.05$) showed defects in invasion, compared with the SR-11 wild-type strain (AJB3). The 35-fold-decreased invasion rate of the *S. typhimurium fim* deletion mutant (AJB4) could be complemented to wild-type (AJB3) levels by introducing the *S. typhimurium fim* operon on a plasmid (pISF101) (Fig. 1F). Therefore, our results confirm a previous report by Horiuchi and coworkers, who suggested that type 1 fimbriae of *S. typhimurium* are necessary for the invasion of HeLa cells (24). We observed that *S. typhimurium* SR-11 and ATCC 14028 differed in their invasiveness for HeLa cells ($P < 0.05$). Introduction of the *S. typhimurium fim* operon on a plasmid (pISF101) increased the invasion rates of strain ATCC 14028. Thus, similar to our results for bacterial adhesion to HeLa cells described above, differences between *S. typhimurium* SR-11 and ATCC 14028 in the invasion of HeLa cells may be due to the levels of expression of their type 1 fimbriae.

A mutation in the *invA* gene resulted in reduced invasion rates but did not affect adhesion to any of the five cell lines tested ($P > 0.1$) (Fig. 1). Therefore, the *S. typhimurium* invasion apparatus mediates entry into a variety of cell types but appears to be unable to discriminate between different cell lines. On the contrary, a defect in invasion of one particular cell line was observed only for a mutant in the fimbrial operon which was necessary for attachment to that cell line. Thus, fimbriae allow *S. typhimurium* to distinguish between different epithelial cell types. In conclusion, our results suggest that the decision as to whether an epithelial cell line is invaded by *S. typhimurium* is made at the level of adhesion. This hypothesis further implies that adhesion determinants other than the *lpf* and *fim* operons are involved during the entry of *S. typhimurium* into Int-407, T-84, and MDCK cells.

S. typhimurium invades an epithelial cell by a mechanism which induces the formation of membrane ruffles on the apical surface of its target cell (17, 21, 27, 41). Several loci involved in entry into epithelial cells have been identified (16, 19, 21, 22, 26, 31, 33, 39). The majority of these loci map between 58 and 60 min on the *S. typhimurium* chromosome to a pathogenicity island (36). The genes located in this region encode the *S. typhimurium* invasion apparatus, which appears to be responsible for the induction of membrane ruffling on the host cell surface. In addition, several genes that are necessary for *S. typhimurium* cell invasion but are located outside this pathogenicity island have been identified. Some of these genes are involved in lipopolysaccharide biosynthesis or bacterial motility and appear to affect invasion indirectly (5, 16, 28). Others, however, may be involved in distinct bacterium-host cell interactions that are specifically required for the invasion phenotype. A two-step model has been proposed for the invasion of epithelial cells; it suggests that *S. typhimurium* attaches to the cell surface through as-yet-unidentified molecules and subsequently initiates a signal transduction cascade (6, 15). The role of fimbrial adhesins during invasion may be to mediate this initial attachment to the host cell surface.

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REFERENCES

1. **Bäumler, A. J., and F. Heffron.** 1995. Identification and sequence analysis of *lpfABCDE*, a putative fimbrial operon of *Salmonella typhimurium*. J. Bacteriol. **177:**2087–2097.
2. **Bäumler, A. J., R. M. Tsolis, F. A. Bowe, J. G. Kusters, S. Hoffmann, and F. Heffron.** 1996. The *pef* fimbrial operon of *Salmonella typhimurium* mediates adhesion to murine small intestine and is necessary for fluid accumulation in the infant mouse. Infect. Immun. **64:**61–68.
3. **Bäumler, A. J., R. M. Tsolis, and F. Heffron.** 1996. The *lpf* fimbrial operon mediates adhesion to murine Peyer's patches. Proc. Natl. Acad. Sci. USA **93:**279–283.
4. **Behlau, I., and S. I. Miller.** 1993. A PhoP-repressed gene promotes *Salmonella typhimurium* invasion of epithelial cells. J. Bacteriol. **175:**4475–4484.
5. **Betts, J., and B. B. Finlay.** 1992. Identification of *Salmonella typhimurium* invasiveness loci. Can. J. Microbiol. **38:**852–857.
6. **Bliska, J. B., J. E. Galán, and S. Falkow.** 1993. Signal transduction in the mammalian cell during bacterial attachment and entry. Cell **73:**903–920.
7. **Carter, P. B., and F. M. Collins.** 1974. The route of enteric infection in normal mice. J. Exp. Med. **139:**1189–1203.
8. **Clark, M. A., M. A. Jepson, N. L. Simmons, and B. H. Hirst.** 1994. Preferential interaction of *Salmonella typhimurium* with mouse Peyer's patch M cells. Res. Microbiol. **145:**543–552.
9. **Clegg, S., S. Hull, R. Hull, and J. Pruckler.** 1985. Construction and comparison of recombinant plasmids encoding type 1 fimbriae of members of the family *Enterobacteriaceae*. Infect. Immun. **48:**275–279.
10. **Clegg, S., B. K. Purcell, and J. Pruckler.** 1987. Characterization of genes encoding type 1 fimbriae of *Klebsiella pneumoniae*, *Salmonella typhimurium*, and *Serratia marcescens*. Infect. Immun. **55:**281–287.
11. **Clegg, S., and D. L. Swenson.** 1994. *Salmonella* fimbriae, p. 105–113. *In* P. Klemm (ed.), Fimbriae: adhesion, genetics, biogenesis, and vaccines. CRC Press, Inc., Boca Raton, Fla.
12. **Duguid, J. P., E. S. Anderson, and I. Campbell.** 1966. Fimbriae and adhesive properties in *Salmonellae*. J. Pathol. Bacteriol. **92:**107–137.
13. **Duguid, J. P., M. R. Darekar, and D. W. F. Wheather.** 1976. Fimbriae and infectivity in *Salmonella typhimurium*. J. Med. Microbiol. **9:**459–473.
14. **Ernst, R. K., D. M. Dombroski, and J. M. Merrick.** 1990. Anaerobiosis, type 1 fimbriae, and growth phase are factors that affect invasion of HEp-2 cells by *Salmonella typhimurium*. Infect. Immun. **58:**2014–2016.
15. **Falkow, S., R. R. Isberg, and D. A. Portnoy.** 1992. The interaction of bacteria with mammalian cells. Annu. Rev. Cell Biol. **8:**333–363.
16. **Finlay, B. B., M. N. Starnbach, C. L. Francis, B. A. D. Stocker, S. Chatfield, G. Dougan, and S. Falkow.** 1988. Identification and characterization of Tn*phoA* mutants of *Salmonella* that are unable to pass through a polarized MDCK epithelial cell monolayer. Mol. Microbiol. **2:**757–766.
17. **Francis, C. L., M. N. Stanbach, and S. Falkow.** 1992. Morphological and cytoskeletal changes in epithelial cells occur immediately upon interaction with *Salmonella typhimurium* grown under low-oxygen conditions. Mol. Microbiol. **6:**3077–3087.
18. **Friedrich, M. J., N. E. Kinsey, J. Vila, and R. J. Kadner.** 1993. Nucleotide sequence of a 13.9 kb segment of the 90 kb virulence plasmid of *Salmonella typhimurium*: the presence of fimbrial biosynthetic genes. Mol. Microbiol. **8:**543–558.
19. **Galán, J. E., and R. Curtiss III.** 1989. Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. Proc. Natl. Acad. Sci. USA **86:**6383–6387.
20. **Giannella, R. A., S. B. Formal, G. J. Dammin, and H. Collins.** 1973. Pathogenesis of salmonellosis: studies on fluid secretion, and morphologic reaction in the rabbit ileum. J. Clin. Invest. **52:**441–453.
21. **Ginocchio, C., J. Pace, and J. E. Galán.** 1992. Identification and characterization of a *Salmonella typhimurium* gene involved in triggering the internalization of salmonellae into cultured epithelial cells. Proc. Natl. Acad. Sci. USA **89:**5976–5980.

22. **Groisman, E. A., and H. Ochman.** 1993. Cognate gene clusters govern invasion of host epithelial cells by *Salmonella typhimurium* and *Shigella flexneri*. EMBO J. **12:**3779–3787.
23. **Hohmann, A. W., G. Schmidt, and D. Rowley.** 1978. Intestinal colonization and virulence of *Salmonella* in mice. Infect. Immun. **22:**763–770.
24. **Horiuchi, S., Y. Inagaki, N. Okamura, R. Nakaya, and N. Yamamoto.** 1992. Type 1 pili enhance the invasion of *Salmonella baenderup* and *Salmonella typhimurium* to HeLa cells. Microbiol. Immunol. **36:**593–602.
25. **Isaacson, R. E., and M. Kinsel.** 1992. Adhesion of *Salmonella typhimurium* to porcine intestinal epithelial surfaces: identification and characterization of two phenotypes. Infect. Immun. **60:**3193–3200.
26. **Jones, B. D., and S. Falkow.** 1994. Identification and characterization of a *Salmonella typhimurium* oxygen-regulated gene required for bacterial internalization. Infect. Immun. **62:**3745–3752.
27. **Jones, B. D., N. Ghori, and S. Falkow.** 1994. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. J. Exp. Med. **180:**15–23.
28. **Jones, B. D., C. A. Lee, and S. Falkow.** 1992. Invasion of *Salmonella typhimurium* is affected by the direction of flagellar rotation. Infect. Immun. **60:**2475–2480.
29. **Jones, G. W., D. K. Rabert, M. Svinarich, and H. J. Whitfield.** 1982. Association of adhesive, invasive, and virulent phenotypes of *Salmonella typhimurium* with autonomous 60-megadalton plasmids. Infect. Immun. **38:**476–486.
30. **Jones, G. W., and L. A. Richardson.** 1981. The attachment to and invasion of HeLa cells by *Salmonella typhimurium*: the contribution of mannose-sensitive and mannose-resistant haemagglutinating activities. J. Gen. Microbiol. **127:**361–370.
31. **Kaniga, K., S. Tucker, D. Trollinger, and J. E. Galán.** 1995. Homologs of the *Shigella* IpaB and IpaC invasins are required for *Salmonella typhimurium* entry into cultured epithelial cells. J. Bacteriol. **177:**3965–3971.
32. **Kusters, J. G., G. A. W. M. Mulders-Kremers, C. E. M. van Doornik, and B. A. M. van der Zeijst.** 1993. Effects of multiplicity of infection, bacterial protein synthesis, and growth phase on adhesion to and invasion of human cell lines by *Salmonella typhimurium*. Infect. Immun. **61:**5013–5020.
33. **Lee, C. A., B. D. Jones, and S. Falkow.** 1992. Identification of a *Salmonella typhimurium* invasion locus by selection for hyperinvasive mutants. Proc. Natl. Acad. Sci. USA **89:**1847–1851.
34. **Lindquist, B. L., E. Lebenthal, P.-C. Lee, M. W. Stinson, and J. M. Merrick.** 1987. Adherence of *Salmonella typhimurium* to small-intestinal enterocytes of the rat. Infect. Immun. **55:**3044–3050.
35. **Lockman, H. A., and R. Curtiss III.** 1992. Virulence of non-type 1-fimbriated and nonfimbriated nonflagellated *Salmonella typhimurium* mutants in murine typhoid fever. Infect. Immun. **60:**491–496.
36. **Mills, D. M., V. Bajaj, and C. A. Lee.** 1995. A 40kb chromosomal fragment encoding *Salmonella typhimurium* invasion genes is absent from the corresponding region of the *Escherichia coli* K-12 chromosome. Mol. Microbiol. **15:**749–759.
37. **Purcell, B. K., J. Pruckler, and S. Clegg.** 1987. Nucleotide sequences of the genes encoding type 1 fimbrial subunits of *Klebsiella pneumoniae* and *Salmonella typhimurium*. J. Bacteriol. **169:**5831–5834.
38. **Stojiljkovic, I., A. J. Bäumler, and F. Heffron.** 1995. Ethanolamine utilization in *Salmonella typhimurium*: nucleotide sequence, protein expression, and mutational analysis of the *cchA cchB eutE eutJ eutG eutH* gene cluster. J. Bacteriol. **177:**1357–1366.
39. **Stone, B. J., C. M. Garcia, J. L. Badger, T. Hassett, R. I. F. Smith, and V. Miller.** 1992. Identification of novel loci affecting entry of *Salmonella enteritidis* into eukaryotic cells. J. Bacteriol. **174:**3945–3952.
40. **Swenson, D. L., and S. Clegg.** 1992. Identification of ancillary *fim* genes affecting *fimA* expression in *Salmonella typhimurium*. J. Bacteriol. **174:**7697–7704.
41. **Takeuchi, A.** 1967. Electron microscope studies of experimental salmonella infection. I. Penetration into the intestinal epithelium by *Salmonella typhimurium*. Am. J. Pathol. **50:**109–136.
42. **Tavendale, A., C. K. H. Jardine, D. C. Old, and J. P. Duguid.** 1983. Haemagglutination and adhesion of *Salmonella typhimurium* to HEp2 and HeLa cells. J. Med. Microbiol. **16:**371–380.

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