2006

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Infect. Immun. **2006, 74(7):4361. DOI: 10.1128/IAI.00154-06.**

INFECTION AND IMMUNITY, July 2006, p. 4361–4365 Vol. 74, No. 7
0019-9567/06/$08.00+0 doi:10.1128/IAI.00154-06

Characterization of a Novel Porin Involved in Systemic *Yersinia enterocolitica* Infection

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Received 30 January 2006/Returned for modification 15 March 2006/Accepted 10 April 2006

Yersinia enterocolitica **is an enteric pathogen capable of causing systemic disease in a murine model. We have identified a novel protein, *s*ystemic *f*actor *p*rotein A (SfpA), conserved in other pathogenic bacteria that is involved in systemic disease. Analysis of bacterial colonization revealed that a Δ*sfpA* strain is defective in mesenteric lymph node colonization. Bioinformatics and functional studies suggest that SfpA is a porin.**

The genus *Yersinia* includes three pathogenic species, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Yersinia pestis*, which share a common tropism for lymphoid tissue. Upon ingestion, *Y. enterocolitica* survives the harsh acidic environment of the stomach and progresses to the terminal ileum. Once there, the bacterium invades the specialized lymphoid tissue of the small intestine known as Peyer's patches through interactions with intestinal M cells (5, 9, 12). Successful colonization of the Peyer's patches can subsequently lead to dissemination to deeper tissues, including the mesenteric lymph nodes (MLN), spleen, and liver. Progression of infection to deeper tissues and pathology of these tissues are most commonly seen in immunocompromised individuals (3, 19).

Several factors that have been shown to be involved in various stages of *Y. enterocolitica* infection have been identified. During early stages of colonization, the outer membrane proteins Inv and YadA allow bacteria to adhere to, colonize, and invade the intestinal epithelium (13, 14, 22–24, 35). The Yops encoded by the virulence plasmid are essential at later stages of yersiniosis, presumably to counteract the immune response. Effectors of this system are injected into host cells to prevent phagocytosis by macrophages and hinder the release of proinflammatory cytokines from macrophages and epithelial and endothelial cells (31).

Previously, an in vivo expression technology screen was conducted to identify genes in *Y. enterocolitica* that are expressed during systemic infection (8). One gene identified in this screen, *sif15*, is not found in either *Y. pseudotuberculosis* or *Y. pestis*, and a strain carrying a mutation in *sif15* was attenuated during intraperitoneal competitive infections with wild-type *Y. enterocolitica*. Furthermore, in vitro analysis demonstrated that *sif-15* transcription is significantly increased at 37°C (8). Based on analysis of the sequence around *sif15* (YE3039), it does not appear to be part of an operon (http://www.sanger.ac.uk/Projects/Y_enterocolitica/). Subsequently, we renamed *sif-15* with the designation *sfpA* (*s*ystemic *f*actor *p*rotein A) in order to avoid confusion with *Salmonella* induced filaments (Sifs). In this study, we show that SfpA is located in the membrane and is necessary for sustained colonization of mesenteric lymph nodes. Furthermore, in vitro data suggest that SfpA forms a pore in the outer membrane when expressed in *Escherichia coli*.

Requirement for SfpA in mesenteric lymph nodes. To further define the role of *sfpA* in virulence, an in-frame deletion of *sfpA* was made in *Y. enterocolitica* strain JB580v (16). Flanking 500-bp regions of *sfpA* were cloned into the suicide plasmid pSR47S and subsequently used to exchange the *sfpA* gene with a kanamycin cassette by allelic exchange, as described previously (21). The absence of *sfpA* was confirmed by Southern blotting and PCR (data not shown), and the Δ*sfpA* mutant was called strain YVM1051. Complementation of the Δ*sfpA* mutation was carried out as follows. To construct the complementing *sfpA* strain, the pSR47S plasmid was engineered with regions carrying the intragenic sequence found between the *putA* gene (YE1933) and a phage tail gene (YE1931) flanking a multiple cloning site (pPutA). *sfpA* was cloned into pPutA at the multiple cloning site, and *sfpA* was recombined by allelic exchange into the *sfpA* mutant strain (YVM1051) at the site between YE1933 and YE1931. The construct was confirmed by Southern blotting and PCR (data not shown). The complementing strain was called YVM1220.

To assess the effect of the Δ*sfpA* mutation on virulence, C57BL/6j mice were orally infected with approximately 1×10^8 Δ*sfpA* bacteria from an overnight culture grown in Luria-Bertani broth at 26°C and compared to mice receiving an analogous inoculation with wild-type *Y. enterocolitica* (JB580v) (16). Infections were done as previously described (4, 11). Subsequently, Peyer's patches, MLN, and spleens were harvested at 1, 3, and 7 days postinfection, and the number of CFU in each tissue was determined. Bacterial colonization at 24 h postinfection was similar in all three tissues between wild-type-infected mice and Δ*sfpA* mutant-infected mice (data not shown). Bacterial loads in the Peyer's patches and spleens were equivalent to those in wild-type-infected tissues at both 3 days and 7 days postinfection (Fig. 1A). However, by day 3 postinfection, a significantly smaller number of bacteria were recovered from the MLN of mice infected with the Δ*sfpA* strain (Fig. 1A). By day 7 postinfection, the number of CFU in the MLN of Δ*sfpA* strain-infected mice remained significantly lower ($P = 0.0079$) than that in wild-type-infected tissues, with

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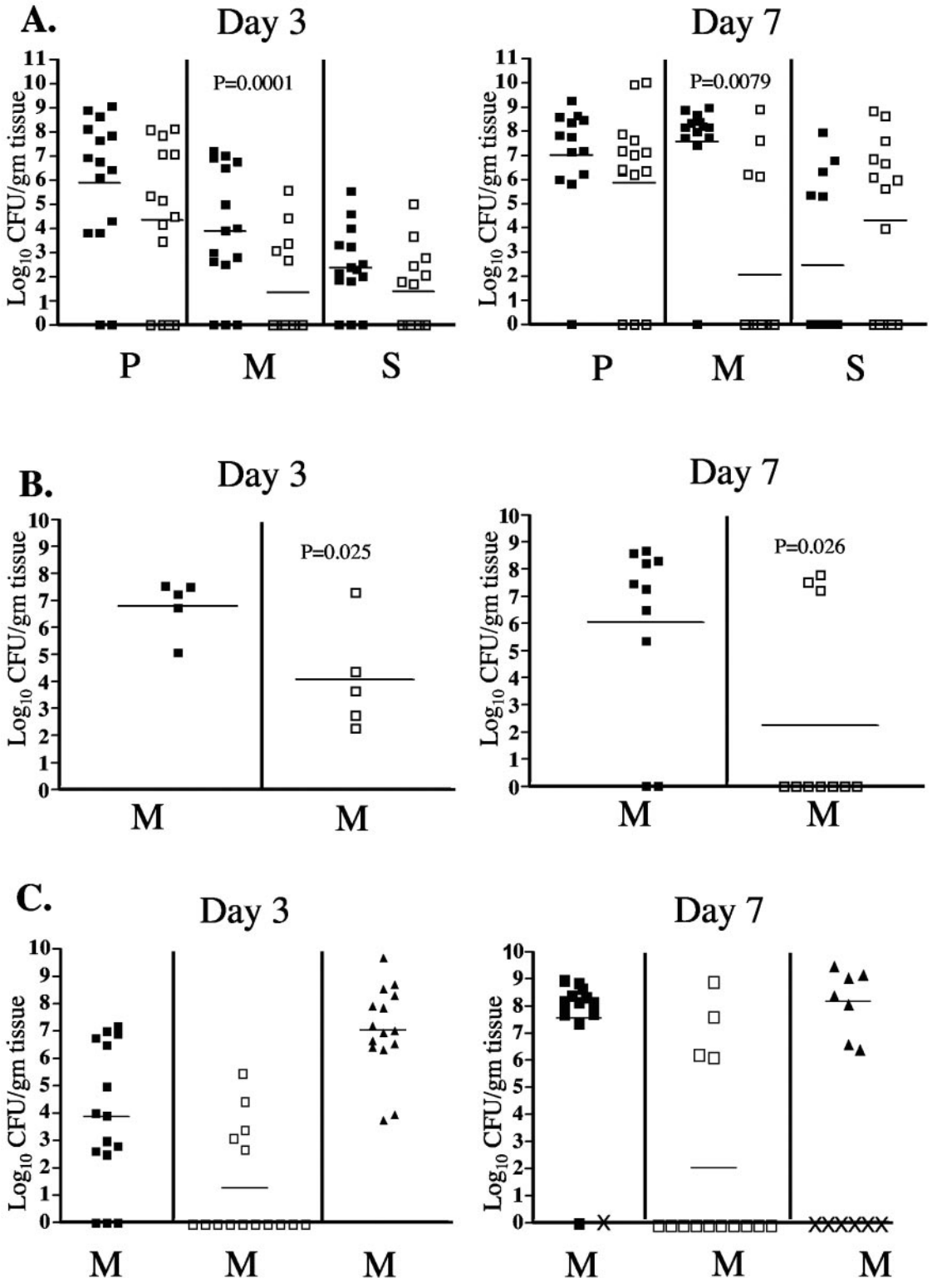

FIG. 1. Bacterial colonization of the mouse by the *sfpA* mutant. (A) C57BL/6j mice were orally infected with 10^8 CFU wild-type *Y. enterocolitica* (JB580v) (■) or the *sfpA* mutant (YVM1051) (□). Mice were sacrificed and organs harvested on day 3 and day 7. The bacterial load for each organ was determined by counting the number of viable bacteria after plating serial dilutions. Results are expressed as CFU per gram of tissue. Each symbol represents an individual mouse, and symbols on the *x* axis were below the limits of detection (limits of detection were as follows: for Peyer's patches, 240; for MLN, 100; and for spleens, 65). The Mann-Whitney test was used to calculate the *P* value. (B) C57BL/6j mice were infected intraperitoneally with 10^4 CFU of wild-type *Y. enterocolitica* (JB580v) (■) or the *sfpA* mutant (YVM1051) (□). The Mann-Whitney test was used to calculate the *P* value. Bacterial loads for mesenteric lymph nodes were determined as described above. (C) C57BL/6j mice were orally infected with 10^8 CFU wild-type *Y. enterocolitica* (JB580v) (■), the *sfpA* mutant (YVM1051) (□), or the complemented *sfpA* mutant strain (YVM1220) (▲). Every "X" on the *x* axis represents one dead mouse; symbols on the *x* axis indicate that the CFU were below the limits of detection. P, Peyer's patch; M, mesenteric lymph node; S, spleen.

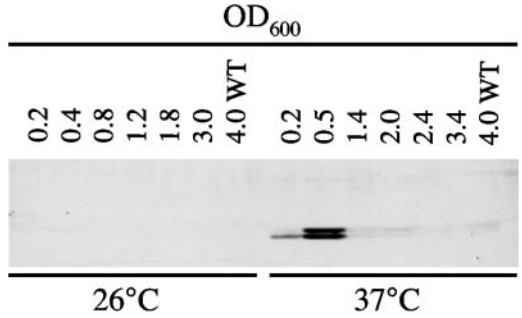

FIG. 2. Levels of $SfpA^{HA}$ in *Y. enterocolitica* during in vitro growth. Cultures of *Y. enterocolitica* $sfpA^{HA}$ (YVM1219) were grown in Luria-Bertani broth at 26°C or 37°C, samples were collected at various time points, and aliquots with equivalent OD_{600} values were loaded into the lanes. Monoclonal anti-HA antibodies (Sigma, St. Louis, MO) were used in immunoblot analysis to determine the levels of SfpA.

10 of the Δ*sfpA* strain-infected mice having no detectable CFU, in contrast to only 1 wild-type-infected mouse with no detectable CFU (limits of detection were as follows: for Peyer's patches, 240 CFU; for MLN, 100 CFU; and for spleens, 65 CFU). These results suggest that SfpA is involved in the establishment, survival, and/or proliferation of *Y. enterocolitica* in the MLN.

The altered progression of infection to the MLN during oral infection with the Δ*sfpA* mutant suggested that this mutant was either defective in dissemination from the Peyer's patches to the MLN or attenuated in its ability to survive in the MLN. To clarify the virulence defect of the Δ*sfpA* mutant, we determined the ability of the mutant to survive in the MLN independent of dissemination from the Peyer's patches by inoculating mice via intraperitoneal injection, thus bypassing the Peyer's patches altogether. C57BL6/j mice were injected with 10^4 CFU of wild-type or Δ*sfpA* bacteria that were grown overnight at 26°C. At 24 hours postinfection, the MLN of all the mice infected with either strain had equivalent levels of colonization (data not shown). At 3 days postinfection, the MLN of all the mice infected with either strain had detectable numbers of bacteria; however, approximately 100-fold fewer bacteria were recovered from tissues of the Δ*sfpA* strain-infected animals (Fig. 1B). By 7 days postinoculation, bacteria were recovered from only 30% of the mice infected with the Δ*sfpA* mutant, in contrast to 80% of the wild-type-infected mice. The complementing strain, YVM1220, completely restored virulence and was able to colonize the MLN to wild-type levels (Fig. 1C). These results indicate that while the Δ*sfpA* mutant could establish infection in the MLN, it was attenuated in its ability to survive in the MLN and was cleared from this tissue at a much higher rate than that for wild-type bacteria. However, these results do not rule out an additional defect in dissemination by the mutant that would result in a smaller number of bacteria migrating from the Peyer's patches to the MLN after oral infection.

In vitro expression of SfpA. SfpA was identified because it was expressed in the host but not in vitro at 26°C. Furthermore, Gort and Miller (8) demonstrated that the transcription of *sfpA* increases in vitro if bacteria are grown at 37°C. To determine the effect of transcriptional regulation on the overall SfpA protein levels in the cell, we generated a Sfp^{HA} epitope-tagged gene fusion and introduced this into the chromosome of *Y. enterocolitica* by homologous recombination. Primers flanking the *sfpA* gene and primers to amplify two hemagglutinin (HA) tags were used to clone a copy of *sfpA* with two C-terminal HA tags into the suicide plasmid pSR47S (21). By homologous recombination, the native *sfpA* gene was replaced with $sfpA^{HA}$ to generate strain YVM1219; substitution of *sfpA* with the fusion construct, $sfpA^{HA}$, was confirmed by Southern blotting and PCR (data not shown). To examine the expression of $SfpA^{HA}$, broth cultures were grown at 26°C and 37°C, and samples were taken periodically. Total protein from each time point was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane (Schleicher and Schuell), and probed with anti-HA antibody (Sigma, St. Louis, MO) as described previously (26). The predicted molecular mass of $SfpA^{HA}$ with its signal sequence is 37 kDa, and that without the signal sequence is 35 kDa. As shown by transcriptional analysis, $SfpA^{HA}$ expression was absent at 26°C. At 37°C, $SfpA^{HA}$ levels peaked during early log phase (optical density at 600 nm [OD_{600}] = 0.5) and appeared to be degraded rapidly over time (Fig. 2). To determine the cellular localization of SfpA, YVM1219 was grown at 37°C overnight and then subcultured the next day for 2 hours. The cellular compartments were fractionated, and protein samples from whole-cell, cytoplasmic, periplasmic, and total membrane frac-

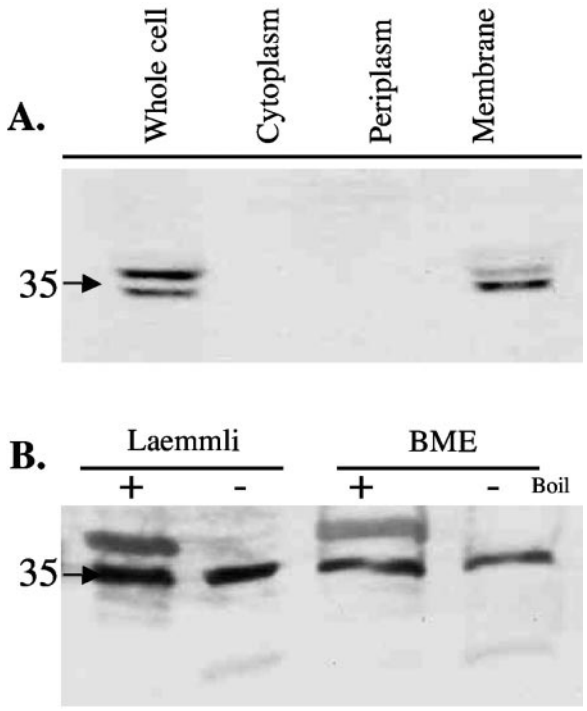

FIG. 3. Localization and heat modifiability of $SfpA^{HA}$ in *Y. enterocolitica*. (A) *Y. enterocolitica* $sfpA^{HA}$ cultures (YVM1219) were grown in Luria-Bertani broth at 37°C to an OD_{600} of 0.5 and fractionated as described previously (17). Monoclonal anti-HA antibodies were used in immunoblot analysis. (B) *Y. enterocolitica* $sfpA^{HA}$ cultures (YVM1219) were grown as described above. Whole-cell lysates were boiled for 10 min in β-mercaptoethanol (BME) or Laemmli buffer (no BME) to rule out any effects of the BME. All samples were run in sodium dodecyl sulfate-polyacrylamide gels and subjected to immunoblot analysis with monoclonal anti-HA antibodies. The 35-kDa molecular mass marker is labeled.

FIG. 4. Sugar diffusion through the SfpA porin. *sfpA* (pSM1) was expressed in the *E. coli* MCR106 *lamB* mutant or in *lamB*$^+$ *E. coli* strain MC4100 and tested for growth on minimal medium in the presence of differently sized sugars. Overnight cultures grown in Luria-Bertani broth were pelleted, washed with M63 salts, and plated on minimal medium containing M63 salts, 10 μM IPTG, and 0.2% (wt/vol) of the indicated sugars. Growth was assessed after 24 to 48 h at 37°C.

tions were analyzed by Western blotting (17). To ensure that proteins did not cross-contaminate fractions, Western blot analysis was performed using antibodies for known *Y. enterocolitica* cytoplasmic (RovA), periplasmic (β-lactamase), and membrane (Inv) proteins (data not shown). SfpA^{HA} was only present in the whole-cell and membrane fractions (Fig. 3A) (17). Since the cellular fractionation results suggest that SfpA is a membrane protein, we wanted to determine if SfpA behaves similarly to other gram-negative outer membrane proteins. One hallmark of outer membrane proteins is that they are heat modifiable, referring to the differences in banding positions and numbers of bands for boiled versus unboiled samples (10, 34). Heat changes the conformation of numerous β-pleated sheets, resulting in altered electrophoretic mobility (15). SfpA^{HA} in *Y. enterocolitica* showed different mobilities in boiled and unboiled samples, further suggesting that SfpA is an outer membrane protein (Fig. 3B).

SfpA appears to be a porin. A protein structure prediction program (http://bmerc-www.bu.edu/index.html) was used to further investigate SfpA (29, 33). In silico analysis of the structure of SfpA predicts the presence of 16 β-pleated sheets in the protein, suggesting the possibility that SfpA is a porin. β-Pleated sheets form β-barrels, which are the main structural components of outer membrane proteins, and porins often consist of β-barrels composed of 16 β-pleated sheets (27). In order to test the prediction that SfpA is a porin, *sfpA* was expressed in *E. coli* MCR106 and tested for growth on minimal medium in the presence of differently sized sugars (18). *E. coli* MCR106 has an internal deletion in the *lamB* gene, which encodes an outer membrane maltoporin that allows sugars to enter the bacterial cell, and therefore it cannot grow on maltose sugars larger than maltotriose unless an alternate porin is expressed (7, 18, 32). The *lamB*$^+$ *E. coli* strain (MC4100) has previously been shown to grow on all sizes of maltosaccharides, from glucose to heptaose (18). Growth of the *lamB* MCR106 strain expressing SfpA on sugars larger than maltotriose would suggest that SfpA can function as a porin by allowing larger sugar nutrients to enter the cell. *sfpA* was cloned into pTrc99A to generate pSM1, allowing expression of *sfpA* from the *trc* promoter by induction with isopropyl-β-D-thiogalactopyranoside (IPTG) (1).

The MCR106 and MC4100 strains carrying either pSM1 or pTrc99A were grown overnight in Luria-Bertani broth, pelleted, washed with M63 salts, and plated on minimal medium containing M63 salts, 10 μM IPTG, and 0.2% (wt/vol) of the indicated sugars (maltosaccharides of increasing size). No growth inhibition was observed in MC4100 expressing SfpA (Fig. 4, row C). MCR106 expressing SfpA grew robustly on sugars up to maltopentaose, but it had only light growth on maltohexaose and maltoheptaose (Fig. 4, row D). In contrast, MCR106 carrying the cloning vector pTRC99A grew robustly on sugars that did not require a dedicated porin such as LamB for uptake but had light growth on plates with sugars larger than maltotriose (Fig. 4, row B) (32). These results suggest that SfpA is able to function as a porin, as evidenced by its increasing the permeability of MCR106 to different carbohydrates.

Conclusions. While the virulence plasmid of *Y. enterocolitica* is necessary for virulence, it is not sufficient, suggesting that there are important virulence factors encoded in the chromosome. *sfpA* is a novel virulence gene carried on the chromo-

some that is involved in the colonization of MLN during acute *Y. enterocolitica* infection. SfpA has amino acid sequence similarity to hypothetical outer membrane proteins in three enteric pathogens, namely, *Salmonella enterica* serovar Typhi, *Escherichia coli* O157:H7, and *Helicobacter pylori* (6, 25, 30). Interestingly, there are no *sfpA* homologous in *Yersinia pestis* or *Yersinia pseudotuberculosis*. While its exact mechanism in virulence is unclear, there are several possible functions for SfpA. Its activity as a porin within the nutrient-limited environment of the host may promote the exchange of nutrients and solutes. Alternatively, SfpA may have functions similar to those of the *Neisseria gonorrhoeae* and *Neisseria meningitidis* porins PorB1A and PorB1B, which induce apoptosis in host cells, act as adjuvants to activate the immune system, and aid in the invasion of epithelial cells (2, 20, 28). Preliminary data suggest that when it is expressed in *E. coli*, SfpA does not act as an adhesin for Chinese hamster ovary cells or confer serum resistance (data not shown). Further studies will focus on determining the mechanism by which SfpA affects the survival of bacteria during systemic infection.

Nucleotide sequence accession number. The nucleotide sequence for *sfpA* has been deposited in GenBank under accession no. DQ470669.

We thank Joseph W. St. Geme III for his experimental and technical advice and Matthew Lawrenz, Chris O'Connor, Clemencia Rojas, and Kim Walker for their critical reviews of the manuscript prior to submission.

This work was supported by research grant AI42736 (V.L.M.).

REFERENCES

1. **Amann, E., B. Ochs, and K. J. Abel.** 1988. Tightly regulated *tac* promoter vectors useful for the expression of unfused and fused proteins in *Escherichia coli*. Gene **69:**301–315.
2. **Bauer, F. J., T. Rudel, M. Stein, and T. F. Meyer.** 1999. Mutagenesis of the *Neisseria gonorrhoeae* porin reduces invasion in epithelial cells and enhances phagocyte responsiveness. Mol. Microbiol. **31:**903–913.
3. **Bouza, E., A. Dominguez, M. Meseguer, L. Buzon, D. Boixeda, M. J. Revillo, L. de Rafael, and J. Martinez-Beltran.** 1980. *Yersinia enterocolitica* septicemia. Am. J. Clin. Pathol. **74:**404–409.
4. **Carter, P. B.** 1975. Pathogenicity of *Yersinia enterocolitica* for mice. Infect. Immun. **11:**164–170.
5. **Clark, M. A., B. H. Hirst, and M. A. Jepson.** 1998. M-cell surface beta1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells. Infect. Immun. **66:**1237–1243.
6. **Deng, W., S. R. Liou, G. Plunkett III, G. F. Mayhew, D. J. Rose, V. Burland, V. Kodoyianni, D. C. Schwartz, and F. R. Blattner.** 2003. Comparative genomics of *Salmonella enterica* serovar Typhi strains Ty2 and CT18. J. Bacteriol. **185:**2330–2337.
7. **Emr, S. D., and T. J. Silhavy.** 1982. Molecular components of the signal sequence that function in the initiation of protein export. J. Cell Biol. **95:**689–696.
8. **Gort, A. S., and V. L. Miller.** 2000. Identification and characterization of *Yersinia enterocolitica* genes induced during systemic infection. Infect. Immun. **68:**6633–6642.
9. **Grutzkau, A., C. Hanski, H. Hahn, and E. O. Riecken.** 1990. Involvement of M cells in the bacterial invasion of Peyer's patches: a common mechanism shared by *Yersinia enterocolitica* and other enteroinvasive bacteria. Gut **31:** 1011–1015.
10. **Hancock, R. E., and A. M. Carey.** 1979. Outer membrane of *Pseudomonas aeruginosa*: heat- 2-mercaptoethanol-modifiable proteins. J. Bacteriol. **140:** 902–910.
11. **Handley, S. A., P. H. Dube, P. A. Revell, and V. L. Miller.** 2004. Characterization of oral *Yersinia enterocolitica* infection in three different strains of inbred mice. Infect. Immun. **72:**1645–1656.
12. **Hanski, C., M. Naumann, H. Hahn, and E. O. Riecken.** 1989. Determinants of invasion and survival of *Yersinia enterocolitica* in intestinal tissue. An *in vivo* study. Med. Microbiol. Immunol. (Berlin) **178:**289–296.
13. **Isberg, R. R., and S. Falkow.** 1985. A single genetic locus encoded by *Yersinia pseudotuberculosis* permits invasion of cultured animal cells by *Escherichia coli* K-12. Nature **317:**262–264.
14. **Isberg, R. R., and J. M. Leong.** 1990. Multiple beta 1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. Cell **60:**861–871.
15. **Kent, N. E., and B. J. Wisnieski.** 1983. Heat modifiability and detergent solubility of outer membrane proteins of *Rhodopseudomonas sphaeroides*. J. Bacteriol. **156:**956–961.
16. **Kinder, S. A., J. L. Badger, G. O. Bryant, J. C. Pepe, and V. L. Miller.** 1993. Cloning of the YenI restriction endonuclease and methyltransferase from *Yersinia enterocolitica* serotype O8 and construction of a transformable R-M+ mutant. Gene **136:**271–275.
17. **Lee, M. H., N. Kosuk, J. Bailey, B. Traxler, and C. Manoil.** 1999. Analysis of F factor TraD membrane topology by use of gene fusions and trypsin-sensitive insertions. J. Bacteriol. **181:**6108–6113.
18. **Marciano, D. K., M. Russel, and S. M. Simon.** 1999. An aqueous channel for filamentous phage export. Science **284:**1516–1519.
19. **Marks, M. I., C. H. Pai, L. Lafleur, L. Lackman, and O. Hammerberg.** 1980. *Yersinia enterocolitica* gastroenteritis: a prospective study of clinical, bacteriologic, and epidemiologic features. J. Pediatr. **96:**26–31.
20. **Massari, P., Y. Ho, and L. M. Wetzler.** 2000. *Neisseria meningitidis* porin PorB interacts with mitochondria and protects cells from apoptosis. Proc. Natl. Acad. Sci. USA **97:**9070–9075.
21. **Merriam, J. J., R. Mathur, R. Maxfield-Boumil, and R. R. Isberg.** 1997. Analysis of the *Legionella pneumophila fliI* gene: intracellular growth of a defined mutant defective for flagellum biosynthesis. Infect. Immun. **65:**2497–2501.
22. **Miller, V. L., and S. Falkow.** 1988. Evidence for two genetic loci in *Yersinia enterocolitica* that can promote invasion of epithelial cells. Infect. Immun. **56:**1242–1248.
23. **Pepe, J. C., and V. L. Miller.** 1993. *Yersinia enterocolitica* invasin: a primary role in the initiation of infection. Proc. Natl. Acad. Sci. USA **90:**6473–6477.
24. **Pepe, J. C., M. R. Wachtel, E. Wagar, and V. L. Miller.** 1995. Pathogenesis of defined invasion mutants of *Yersinia enterocolitica* in a BALB/c mouse model of infection. Infect. Immun. **63:**4837–4848.
25. **Perna, N. T., G. Plunkett III, V. Burland, B. Mau, J. D. Glasner, D. J. Rose, G. F. Mayhew, P. S. Evans, J. Gregor, H. A. Kirkpatrick, G. Posfai, J. Hackett, S. Klink, A. Boutin, Y. Shao, L. Miller, E. J. Grotbeck, N. W. Davis, A. Lim, E. T. Dimalanta, K. D. Potamousis, J. Apodaca, T. S. Anantharaman, J. Lin, G. Yen, D. C. Schwartz, R. A. Welch, and F. R. Blattner.** 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature **409:**529–533.
26. **Sambrook, J. E., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
27. **Schulz, G. E.** 2002. The structure of bacterial outer membrane proteins. Biochim. Biophys. Acta **1565:**308–317.
28. **Sparkes, B. G.** 1983. Immunomodulating activity of meningococcal antigens. Can. J. Microbiol. **29:**1611–1618.
29. **Stultz, C. M., J. V. White, and T. F. Smith.** 1993. Structural analysis based on state-space modeling. Protein Sci. **2:**305–314.
30. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:** 539–547.
31. **Trulzsch, K., T. Sporleder, E. I. Igwe, H. Russmann, and J. Heesemann.** 2004. Contribution of the major secreted Yops of *Yersinia enterocolitica* O:8 to pathogenicity in the mouse infection model. Infect. Immun. **72:**5227–5234.
32. **Wandersman, C., M. Schwartz, and T. Ferenci.** 1979. *Escherichia coli* mutants impaired in maltodextrin transport. J. Bacteriol. **140:**1–13.
33. **White, J. V., C. M. Stultz, and T. F. Smith.** 1994. Protein classification by stochastic modeling and optimal filtering of amino-acid sequences. Math. Biosci. **119:**35–75.
34. **Wilson, M. E.** 1991. The heat-modifiable outer membrane protein of *Actinobacillus actinomycetemcomitans*: relationship to OmpA proteins. Infect. Immun. **59:**2505–2507.
35. **Yang, Y., and R. R. Isberg.** 1993. Cellular internalization in the absence of invasin expression is promoted by the *Yersinia pseudotuberculosis yadA* product. Infect. Immun. **61:**3907–3913.

Editor: J. B. Bliska