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# Naturally Occurring Deletions in the Centisome 63 Pathogenicity Island of Environmental Isolates of *Salmonella* spp.

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**We have identified several environmental isolates of *Salmonella senftenberg* and *S. litchfield* which carry a deletion encompassing a vast segment of the centisome 63 region of the *Salmonella* chromosome. The deletion includes the entire *inv*, *spa*, and *hil* loci, which are required for entry of *Salmonella* spp. into mammalian cells. Consequently, these isolates were found to be markedly deficient in the ability to enter cultured epithelial cells. In contrast, no deletions were found in the corresponding regions of the chromosomes of clinical isolates of these serovars; consequently, these isolates were found to be highly invasive for cultured epithelial cells. These data confirm the importance of the centisome 63 region of the *Salmonella* chromosome in mediating the entry of these organisms into cultured mammalian cells and indicate that additional entry pathways are presumably not utilized by these environmental isolates. These results are also consistent with the notion that this region constitutes a pathogenicity island which remains unstable in certain *Salmonella* serotypes.**

The ability of *Salmonella* spp. to adhere to and enter the cells of the intestinal epithelium is an essential step in the pathogenic life cycle of these organisms. This event is the consequence of a two-way interaction between *Salmonella* spp. and their host cells that results in the initiation of host cell signaling pathways which ultimately lead to membrane ruffling, cytoskeletal rearrangements, and internalization of the bacteria (reviewed in reference 8). Studies of the molecular genetic bases of this interaction have led to the identification of several genetic loci whose gene products facilitate the entry of these organisms into cultured epithelial cells. Most of these entry loci map to the centisome 63 region of the *Salmonella* chromosome and encode a specialized type III protein secretion system. This protein secretion system directs the export and translocation into the host cell of a number of proteins which ultimately trigger the signaling events that lead to bacterial internalization (reviewed in reference 9).

The importance of this chromosomal region in different *Salmonella* serovars is supported by two studies that examined the presence and functionality of *invA*, a gene that encodes an essential component of the invasion-associated protein secretion apparatus (12). Colony and Southern blot analyses determined that *invA* was present in over 100 different invasive strains representing 30 different serovars of *Salmonella*. Studies conducted with several representative serovars of *Salmonella*, including *Salmonella typhi*, indicated that the *invA* gene is not only present but also required for entry of *Salmonella* spp. into cultured epithelial cells (11). A subsequent, more extensive study assessed the presence of the *invA* gene in a collection of 630 strains representing over 100 different serovars of *Salmonella* that had been isolated from environmental sources, animals, and humans (24). With the exception of two isolates of *S. senftenberg* and two isolates of *S. litchfield*, all *Salmonella* strains tested positive for the presence of *invA*. These data indicated that the *inv* locus is widely distributed in a large number of *Salmonella* serotypes. Interestingly, the strains that were shown to lack *invA* sequences in this study had been recovered from environmental samples and were not specifically associated with disease (24). The deletion of the *invA* gene in these environmental isolates implied that these isolates would be deficient for entry into cultured cells and would therefore display reduced virulence. Conversely, these isolates may utilize other entry pathways independent of *invA*. Multiple entry pathways have been identified in other pathogens, such as *Yersinia* spp. (2, 20, 28) and *Listeria monocytogenes* (27).

We therefore expanded our study in order to determine if the *invA* deletion was a common occurrence among environmental isolates of these two serovars and to establish whether these *Salmonella* serovars utilize alternative pathways of entry independent of that encoded at centisome 63.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, media, and growth conditions.** The wild-type *S. typhimurium* strain SR11 (25), its *invA* isogenic derivative strain SB147 (12), and the *invH* strain SB150 (1) have been previously described. *S. typhimurium* GW1702, which carries a Tn*10* insertion in the *mutS* gene, has been described elsewhere (23). The *S. senftenberg* and *S. litchfield* isolates were obtained from the *Salmonella* Laboratory at the Health of Animals Laboratory (Guelph, Ontario, Canada), the Laboratory Centre for Disease Control (Ottawa, Ontario, Canada), and the Ontario Ministry of Health (Toronto, Ontario, Canada). The nine environmental isolates of *S. litchfield* that lack *invA* by PCR analysis were from either swabs or litter from healthy turkeys. Of the remaining 11 *S. litchfield* isolates (*invA*$^+$ by PCR analysis), 10 were from human sources and 1 was isolated from shrimp implicated in a *Salmonella* outbreak. All *S. senftenberg* isolates were recovered from poultry feed. Strains were grown in L broth or on L-agar plates. When appropriate, antibiotics were added to the growth medium at the following concentrations: kanamycin, 30 μg/ml; ampicillin, 100 μg/ml, and tetracycline, 12 μg/ml.

**In vitro tissue culture adherence and invasion assays.** Adherence and invasion assays using Henle-407 cells grown to 80% confluence in 24-well tissue culture dishes were performed as previously described (10).

**Genetic techniques, DNA isolation, probe preparation, and Southern hybridization.** Linkage mapping of the *inv* locus in the different *S. litchfield* and *S. senftenberg* strains was carried out by P22HT*int*-mediated transduction as described previously (12). Isolation of total cell DNA and Southern hybridization were carried out as described elsewhere (11). DNA probes used in these studies (Fig. 1) were prepared as follows. Probe 1 was prepared from pSB151 (12) by restriction with *Bgl*II to yield an 800-bp internal fragment of *invA*. Probe 2 was prepared by digesting pSB710 (22a) with *Not*I to yield a DNA fragment that

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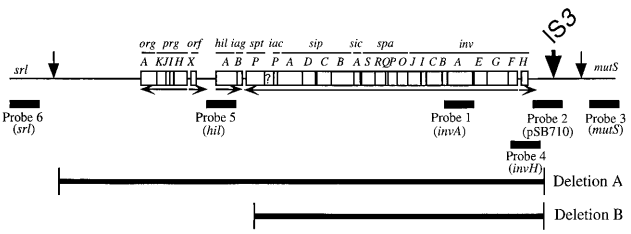


FIG. 1. DNA probes used in Southern hybridization analysis and schematic representation of the chromosomal deletions in environmental isolates of *S. senftenberg* and *S. litchfield*. Probes are indicated in relation to the centisome 63 region of the *S. typhimurium* chromosome and are not drawn to scale. Vertical arrows indicate the estimated boundaries of the pathogenicity island and the locations of the IS*3*-like sequences. Deletion A corresponds to *S. senftenberg* isolates 834 and 856 and all *invA* isolates of *S. litchfield*; deletion B corresponds to *S. senftenberg* isolate 16. The exact boundaries of the deletions have not been mapped, and the sizes are approximations and not drawn to scale.

contains sequences starting 1 kb from *invH* and spanning a region 5 kb downstream from this gene which includes one of the ends of the centisome 63 pathogenicity island. Probe 3 was prepared by digesting pGW1811 (23) with *Pvu*II to yield a 2.1-kb fragment containing internal sequences of the *mutS* gene. Probe 4 was generated by digesting pSB300 (1) with *Dra*I and *Pst*I to yield a 528-bp fragment containing sequences internal to *invH*. Probe 5 was generated by digesting pVB3 with *Xho*I to yield an internal 1.3-kb fragment of the *hil* invasion locus (21). Probe 6 was prepared by digesting pEE526 with *Cla*I and *Bam*HI to yield a 1.7-kb fragment of *S. typhimurium* sequence immediately adjacent to the *srl* operon (21). The resulting DNA fragments were separated by electrophoresis on a 0.7% agarose gel, and the DNA fragments of interest were isolated by using Gene Clean II (Bio 101, La Jolla, Calif.). Detection of *invA* by PCR in the different *Salmonella* strains was carried out with primers complementary to sequences 5′ (5′GTGAAATTATCGCCACGTTCGGGCAA3′) and 3′ (5′TCATCGCACCGTCAAAGGAACC3′) of this gene as described previously (24). The IS*3* probe was generated by PCR with total cell DNA from *S. choleraesuis* as a template and primers (5′GGAATTCTGAACTTACTCAGCA ATAG3′ and 5′GGAATTCAGCTGGTAGAGCTATGA3′) derived from the previously identified IS*3* sequence located 394 bp downstream from the *invH* gene in *S. choleraesuis* (1). All DNA probes were denatured by boiling for 5 min and labeled with [α-$^{32}$P]ATP (Amersham Corp., Arlington Heights, Ill.), using a random primer DNA labeling kit (Bethesda Research Laboratories, Gaithersburg, Md.). Membranes were blotted and exposed to X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.). The membranes were subsequently stripped by boiling for 30 min in 1% sodium dodecyl sulfate–1 mM EDTA–10 mM Tris-HCl (pH 7.5); to verify the efficiency of the stripping, they were exposed to an X-ray film. The process was subsequently repeated with other probes.

## RESULTS

**Environmental isolates of *S. litchfield* and *S. senftenberg* carry a deletion in the *inv* locus.** The absence of *invA* in the environmental isolates of *S. senftenberg* and *S. litchfield* prompted us to examine additional environmental isolates of these two serovars for the presence of *invA*. Initially, these isolates were screened by PCR for the presence of *invA*. Subsequently, the presence or absence of *invA* in all of the isolates was confirmed by Southern blot analysis with an internal fragment of *invA* from *S. typhimurium* serving as a probe. As shown in Table 1 and Fig. 2, several environmental isolates of *S. senftenberg* and *S. litchfield* lacked sequences that could be amplified with primers complementary to *invA* or that could hybridized to an *invA* probe. All *invA*$^+$ isolates showed hybridization when tested with an internal fragment of *invA*, thereby corroborating the PCR analysis.

**Inv$^-$ strains of *S. litchfield* and *S. senftenberg* are deficient for entry into cultured epithelial cells.** We examined the *inv* isolates of *S. litchfield* and *S. senftenberg* for the ability to attach to and enter cultured epithelial cells. Absence of *invA* should result in an entry-deficient phenotype since the *inv* pathway has been shown to be essential in all *Salmonella* isolates previously tested (11). Conversely, entry proficiency among the *invA* environmental isolates would indicate the presence of an alternative entry pathway. As shown in Table 1, all *invA*$^+$ and *invA* strains were capable of binding to cultured Henle-407 cells at levels comparable to that of the wild-type strain *S. typhimurium* SR11. Only the *invA S. litchfield* 885 isolate showed an approximately 10-fold decrease in its ability to bind to the same cells. All *invA*$^+$ strains were capable of entering Henle-407 cells at levels comparable to those of *S. typhimurium* SR11. On the contrary, *invA* isolates showed 100- to 2,000-fold decreases in the entry levels. These values are similar to that of *S. typhimurium* SB147, which carries a defined, nonpolar mutation in *invA*. Together, these data indicate that the loss of the *invA* gene results in an entry-deficient phenotype and that no alternative entry pathway is therefore utilized by these isolates.

TABLE 1. Adherence to and invasion of cultured Henle-407 cells by wild-type and *invA* isolates of *S. senftenberg* and *S. litchfield*

| Strain | Serovar | PCR analysis[a] | Mean ± SD ($n$ = 3)[b] | |
|---|---|---|---|---|
| | | | % Adherence[c] | % Internalization[d] |
| SR11 | *S. typhimurium* | ND | 93 ± 4 | 88 ± 2 |
| SB147 | *S. typhimurium* | ND | 85 ± 3 | 0.09 ± 0.001 |
| 58 | *S. senftenberg* | *invA*$^+$ | 29 ± 3 | 22 ± 1 |
| 149 | *S. senftenberg* | *invA*$^+$ | 60 ± 1 | 39 ± 2 |
| 16 | *S. senftenberg* | *invA* | 36 ± 3 | 0.06 ± 0.01 |
| 834 | *S. senftenberg* | *invA* | 59 ± 1 | 0.02 ± 0.002 |
| 856 | *S. senftenberg* | *invA* | 61 ± 3 | 0.005 ± 0.002 |
| 886 | *S. litchfield* | *invA*$^+$ | 53 ± 0.3 | 38 ± 2 |
| 887 | *S. litchfield* | *invA*$^+$ | 49 ± 1 | 45 ± 3 |
| 888 | *S. litchfield* | *invA*$^+$ | 89 ± 6 | 56 ± 2 |
| 889 | *S. litchfield* | *invA*$^+$ | 65 ± 3 | 68 ± 4 |
| 890 | *S. litchfield* | *invA*$^+$ | 64 ± 5 | 51 ± 2 |
| 891 | *S. litchfield* | *invA*$^+$ | 55 ± 6 | 55 ± 4 |
| 892 | *S. litchfield* | *invA*$^+$ | 75 ± 4 | 50 ± 2 |
| 894 | *S. litchfield* | *invA*$^+$ | 66 ± 3 | 47 ± 4 |
| 895 | *S. litchfield* | *invA*$^+$ | 62 ± 6 | 48 ± 1 |
| 896 | *S. litchfield* | *invA*$^+$ | 83 ± 3 | 46 ± 2 |
| 897 | *S. litchfield* | *invA*$^+$ | 99 ± 4 | 96 ± 2 |
| 871 | *S. litchfield* | *invA* | 35 ± 2 | 0.036 ± 0.003 |
| 879 | *S. litchfield* | *invA* | 29 ± 0.5 | 0.02 ± 0.004 |
| 880 | *S. litchfield* | *invA* | 44 ± 2 | 0.7 ± 0.004 |
| 881 | *S. litchfield* | *invA* | 30 ± 1 | 0.01 ± 0.003 |
| 883 | *S. litchfield* | *invA* | 45 ± 1 | 0.01 ± 0.002 |
| 884 | *S. litchfield* | *invA* | 51 ± 3 | 0.01 ± 0.003 |
| 885 | *S. litchfield* | *invA* | 5 ± 0.3 | 0.006 ± 0.003 |
| 893 | *S. litchfield* | *invA* | 42 ± 2 | 0.06 ± 0.004 |
| 898 | *S. litchfield* | *invA* | 44 ± 2 | 0.035 ± 0.005 |

[a] Carried out with primers complementary to sequences from the *invA* gene as indicated in Materials and Methods. ND, not determined.
[b] Similar results were observed in several repetitions of these experiments.
[c] Percentage of the bacterial inoculum associated to cultured Henle-407 cells 2 h after infection.
[d] Percentage of the bacterial inoculum that survived gentamicin treatment.

**Mapping of the locations and extents of the deletions of the invasion-associated pathogenicity island of *S. litchfield* and *S. senftenberg* strains.** We first mapped the location of the *inv* locus in the chromosome of each of the various clinical isolates of *S. litchfield* and *S. senftenberg*. We constructed derivatives *S. litchfield* isolates 886 and 887 and of *S. senftenberg* isolate 58 carrying insertions of an *aphT* cassette (encoding kanamycin resistance) in the *invH* gene. We then investigated the linkage of these insertions to the *mutS*::Tn*10* allele of strain GW1702 by P22HT*int*-mediated transduction. This allele, located at centisome 63 of the *Salmonella* chromosome, constitutes the *invH*-proximal border of the invasion-associated pathogenicity island and has previously been shown to be linked to the *inv*

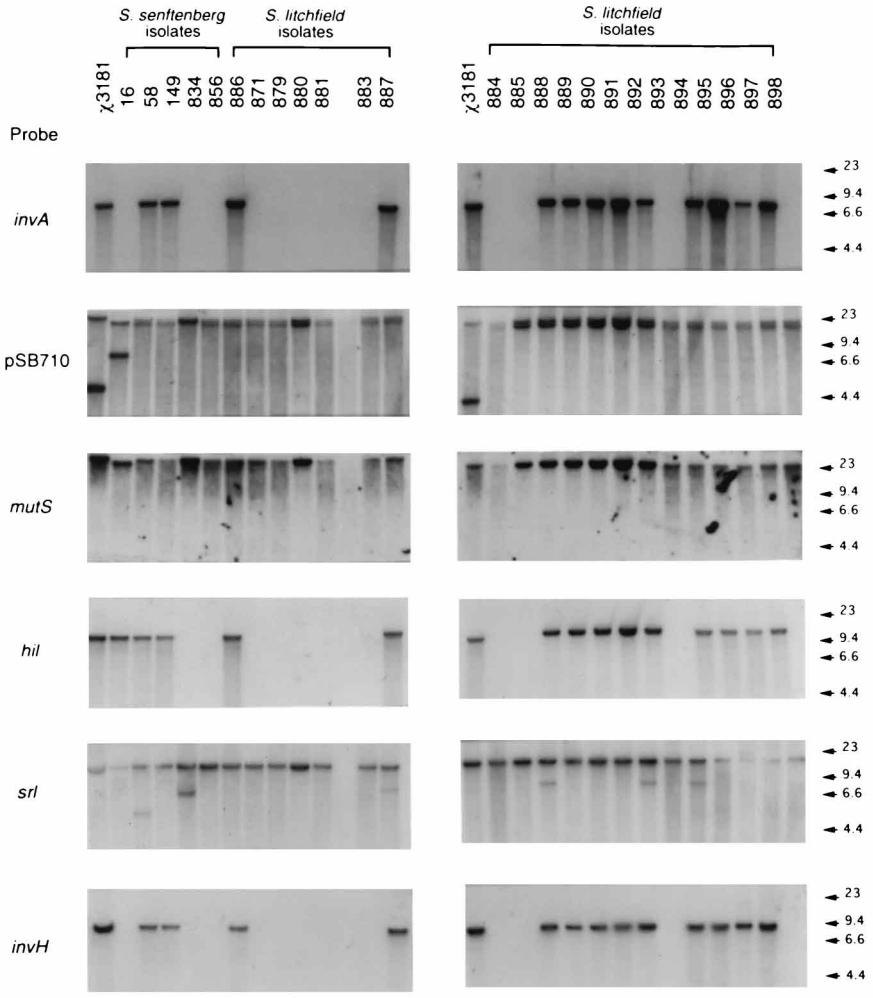


FIG. 2. Southern analyses of total cell DNAs of isolates of *S. senftenberg* and *S. litchfield*. Lanes contain total cell DNAs isolated from *S. typhimurium* SR11 and from the *S. senftenberg* and *S. litchfield* isolates characterized in Table 1, digested with *Eco*RI. Probes used for the blots are indicated to the left. The arrows to the right indicate the positions of the *Hin*dIII-digested lambda size markers in kilobases.

locus. The *invH* genes in all of these strains were found to be similarly linked to *mutS* (actual linkage values: *S. litchfield* 886, 29%; *S. litchfield* 887, 28%; and *S. senftenberg* 58, 27%), therefore establishing that in *S. litchfield* and *S. senftenberg*, the invasion-associated pathogenicity island is also located at centisome 63.

To estimate the extent of the chromosomal deletion in each of several environmental isolates of *S. senftenberg* and *S. litchfield* that did not hybridize with the *invA* probe, we tested the ability of the cosmid clone pYA2217 to complement the entry defect of these organisms. pYA2217 contains a fragment of the *S. typhimurium* chromosome that includes the *inv* and *spa* loci (10). Introduction of pYA2217 into *S. senftenberg* 16, 834, and 856 and *S. litchfield* 871, 879, 885, and 898 did not restore to these isolates the ability to enter cultured cells (data not shown). These data suggested that the deletions encompass a region which extends beyond the *inv* and *spa* loci.

To determine the extents of the deletions, Southern hybridization analyses with probes encompassing the entire centisome 63 region of the *Salmonella* chromosome were performed. Three different probes were used to map the extents of the deletions in regions upstream of the *invA* gene: a 5-kb fragment from pSB710 (22a), which contains sequences spanning approximately 6 kb upstream from *invH* (Fig. 1, probe 2); a 2.1-kb *Pvu*II fragment of plasmid pGW1811 (Fig. 1, probe 3) encoding the *mutS* gene (23), which constitutes one of the borders of the invasion region; and a 523-kb fragment of pSB300 (1) containing sequences of the *invH* gene (Fig. 1, probe 6). To define the boundaries of the deletions in the region downstream from *invA*, blots were probed with an internal 1.3-kb *Xho*I fragment of pVB3 (Fig. 1, probe 4) containing the *hil* invasion locus and a 1.7-kb fragment of pEE526 (Fig. 1, probe 5) which contains sequences flanking the *srl* locus, which constitutes the other boundary of the centisome 63 pathogenicity island.

None of the *invA* strains showed hybridization to the *invH* probe or, with the exception of *S. senftenberg* 16, to the *hil* probe (Fig. 2). The *mutS* gene and the *Not*I fragment of pSB710 containing sequences downstream of *invH* were present in all strains tested (Fig. 2). The *srl* gene probe hybridized to all strains tested (Fig. 2), which is consistent with the fact that all of these isolates, with the exception of *S. litchfield* 896 ($invA^+$), were able to ferment sorbitol (data not shown). The inability of *S. litchfield* 896 to ferment sorbitol is not due to a deletion in the *srl* locus but is most likely due to a point

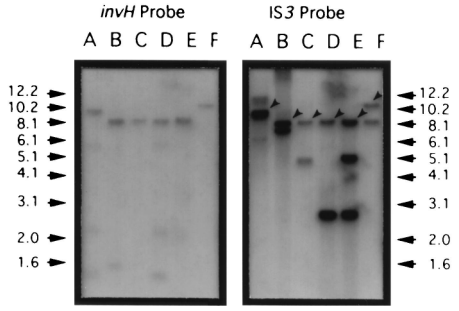


FIG. 3. Localization of IS*3*-homologous sequences in the vicinity of *invH*. Total cell DNAs of *S. senftenberg* 58 (lanes A and D), *S. litchfield* 886 (lanes B and E), and *S. choleraesuis* (lanes C and F) were digested with *Pst*I (lanes A to C) and *Eco*RI (lanes D to F) and probed with internal sequences from both *invH* and IS*3*, as indicated. Arrows to the left and right denote the molecular weight standards in kilobases. Arrows above bands indicate DNA fragments which contain both *invH* and IS*3*-homologous sequences.

mutation in one of the sorbitol utilization genes, as this strain hybridized with a probe containing the *srl* genes.

The restriction patterns obtained with probes hybridizing to sequences upstream of *invA* and the size analysis of the 25-kb region upstream of *invA* indicate that the deletions end within 1 kb downstream of the *invH* gene (Fig. 1 and 2). With the exception of *S. senftenberg* 16, the deletions extend downstream of *invA* through at least part of the *hil* locus but end prior to the *srl* gene, as indicated by the restriction patterns obtained with probes hybridizing to sequences in the *hil* and *srl* loci (Fig. 2). The deletion in *S. senftenberg* 16 appears to end in approximately the same region of the *mutS* side of the pathogenicity island but does not encompass the *hil* region (Fig. 2). No restriction fragment length polymorphisms were detected between the wild-type *S. typhimurium* strain and the *invA*$^+$ isolates of *S. senftenberg* and *S. litchfield* when probed with *invA*, *hil*, and *invH* sequences (Fig. 2). These data indicate that the *inv* and *hil* regions are highly conserved among invasive isolates of these three serovars.

**Identification of IS*3*-homologous sequences bordering the *inv* region in invasive isolates of *S. senftenberg* and *S. litchfield*.** The observation of chromosomal deletions in environmental isolates of *S. litchfield* and *S. senftenberg* indicates a genetic instability of the centisome 63 region of the chromosome in these serovars. The presence of insertion sequences or direct repeats has been associated with genetically unstable regions of the chromosome in a number of pathogens (3, 7, 16). Previous studies have shown that sequences downstream of the *invH* gene of *S. choleraesuis* have significant similarity to the insertion sequence IS*3* (1). Therefore, we examined the *S. litchfield* and *S. senftenberg* strains for the presence of IS*3*-homologous sequences in the vicinity of *invH*. Total cell DNAs from *S. senftenberg* isolate 58 (*invA*$^+$), *S. litchfield* isolate 886 (*invA*$^+$), and *S. choleraesuis* (as a positive control) were digested with various restriction enzymes and probed with sequences containing internal fragments of *invH* and IS*3*. As shown in Fig. 3, using *Pst*I and *Eco*RI digests, we were able to colocalize an IS*3* sequence and the *invH* gene to common DNA fragments in all three strains. Based on fragment size and sequence analysis of the *inv* locus, we have determined that the IS*3*-homologous sequences are located ~1 kb and ~500 bp downstream from the end of *invH* in *S. senftenberg* 58 and *S. litchfield* 886, respectively. The locations of the IS*3* sequences in the *S. litchfield* and *S. senftenberg* isolates examined resemble *S. choleraesuis*, where the IS*3* sequence is located 337 bp downstream from the termination codon of *invH* (1).

## DISCUSSION

Through PCR and Southern analyses, we have identified environmental isolates of *S. senftenberg* and *S. litchfield* that have naturally occurring deletions in the centisome 63 region of the *Salmonella* chromosome. With the exception of *S. senftenberg* 885, all the isolates carrying a deletion in this region were able to adhere to cultured epithelial cells at levels comparable to those of wild-type strains. In contrast, these isolates were markedly (100- to 2,000-fold) deficient in the ability to enter cultured epithelial cells compared to the PCR *invA*$^+$ isolates of the same serovars and a wild-type strain of *S. typhimurium*. This deficiency is comparable to the reduced level of entry seen in strains of *Salmonella* carrying defined mutations in genes of the *inv* or *spa* locus (4–6, 12, 13, 15). These results therefore indicate that this region is essential for the entry phenotype, and this in turn implies that these strains would be avirulent in their host species. Recently, another pathogenicity island has been identified in *S. typhimurium* (22, 26). Although we have not investigated the presence of this second pathogenicity island in the isolates of *S. senftenberg* and *S. litchfield*, our data indicate that no alternative host cell entry pathways are encoded in these isolates.

The inability of pYA2217 to complement the entry defect suggested that the deletions encompass regions beyond *inv* and *spa*, since both loci are encoded in this plasmid (10). To determine the extents of the deletions, Southern hybridization analyses were carried out with various probes containing sequences from regions both upstream and downstream of *invA* (Fig. 1). Although the exact boundaries of each deletion have not been precisely established, it is clear that they encompass large portions of the centisome 63 region of the chromosome and that with the exception of *S. senftenberg* 16, the extents of the deletions are very similar (Fig. 2). Southern hybridization and restriction analyses indicate that these deletions end within 1 kb downstream from the *invH* gene and that they extend downstream of *invA* through the *hil* invasion locus ending prior to the *srl* genes. Presumably, they encompass most of the centisome 63 pathogenicity island (21). Although the entry-deficient phenotype of *S. senftenberg* 16 is comparable to that of the other PCR *invA* isolates, the deletion of this isolate is more limited and does not include the *hil* region.

It is now clear that the centisome 63 region of the *Salmonella* chromosome constitutes another example of a pathogenicity island (21). The unusual composition of its DNA sequence led to the proposition that this region must have been acquired by horizontal transmission from a foreign ancestor relatively recently in evolution, as the DNA sequence has not had time to ameliorate (12, 13). The concept of pathogenicity islands has emerged from studies of a number of pathogens which have revealed large virulence-associated regions seemingly acquired in block (3, 14, 16, 19). As recently proposed, the genes encoded in these regions of the chromosome may constitute examples of "selfish operons" (17).

Often, sequences resembling mobile DNA elements have been identified in the vicinity of these pathogenicity islands. For example, uropathogenic *Escherichia coli* strains contain two pathogenicity islands (PAI I and II) that encode hemolysin and P-related fimbriae (3, 16, 18). In a number of enteric species, IS*1* sequences have been found flanking the genes encoding the synthesis and transport of the siderophore aerobactin. The precise excision of these pathogenicity islands, which is often observed in these strains, appears to be related

to the presence of these insertion sequences or short direct repeats at the ends of each DNA segment that are involved in site-specific recombination (3, 7, 16). Spontaneous deletions of large regions of the bacterial chromosome of *Yersinia pestis* lead to the lack of expression of a number of virulence traits, including the production of the iron-repressible outer membrane proteins, or Irps. Repetitive elements considered to be IS*100* were found at both ends of the deletions and are considered responsible for the deletions (7). Therefore, the presence of mobile DNA elements in relation to pathogenicity islands could account not only for the evolutionary acquisition of these virulence systems among a diverse group of pathogens but also for their intrinsic instability. Sequences similar to the insertion sequence IS*3* have been previously identified in the vicinity of the *invH* gene of *S. choleraesuis*, which is located toward one of the ends of the invasion-associated pathogenicity island of this organism. Similarly, we have found IS*3*-homologous sequences at similar locations in the chromosomes of clinical isolates of *S. senftenberg* and *S. litchfield*. Through Southern hybridization analysis, we were able to colocalize an IS*3*-homologous sequence and the *invH* gene to common DNA fragments. Although the presence of IS*3*-like sequences on the other boundary of the invasion-associated pathogenicity island of *Salmonella* has not been determined, it is possible that the presence of these sequences contributes to the genetic instability of this region in these *Salmonella* serovars.

Microbial pathogens have the ability to adapt to changes in the environment by careful regulation of the expression of different virulence determinants. Thus, the expression of different virulence genes is either activated or repressed in different environments or during different stages of the pathogenic cycle. In addition, pathogens can rapidly adapt to more permanent alterations in their environment, in many instances resulting in significant changes in their pathogenic cycle. Often, this adaptation is the result of the acquisition or loss of genetic information. The high incidence of *Salmonella* in commercial layer flocks in combination with modern poultry-raising methods could create a high probability of a continual fecal-oral infection cycle. In this instance, the rapid and recurring passage of *Salmonella* through the intestinal tract of the host may not require that the organisms enter the cells of the intestinal epithelium in order to achieve successful replication. In such an environment, the presence of the *inv* genes may no longer confer a selective advantage, and they may tend to disappear form the population, particularly if present in a genetically unstable region of the chromosome, as appears to be the case in isolates of *S. senftenberg* and *S. litchfield*.

In summary, we have identified isolates of *S. senftenberg* and *S. litchfield* that are not associated with disease and that have large deletions in the invasion-associated region of their chromosomes at centisome 63. These results indicated that this pathogenicity island remains unstable in certain *Salmonella* serovars and may have implications for the epidemiology as well as for eradication and prevention programs.

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