A chromosomal region surrounding the ompD porin gene marks a genetic difference between *Salmonella typhi* and the majority of *Salmonella* serovars

Carlos A. Santiviago, Cecilia S. Toro,† Sergio A. Bucarey and Guido C. Mora

In this work it is shown that the majority of *Salmonella* serovars most frequently associated with the systemic infection of vertebrate hosts produce a major outer-membrane porin, OmpD. However, OmpD is absent from the outer-membrane protein profiles of *Salmonella typhi* strain Ty2 and 26 clinical isolates of *S. typhi* examined by SDS-PAGE. To determine whether the *ompD* gene is present in *S. typhi*, primers internal to the *ompD* coding sequence were used to amplify the gene by PCR. With the exception of *S. typhi* strains, the *ompD* gene was amplified from the genomes of all *Salmonella* serovars tested. Consistently, a specific *ompD* probe did not hybridize with DNA isolated from the *S. typhi* strains. Taken together, these results demonstrate that *S. typhi* does not produce OmpD due to the absence of the *ompD* gene. Furthermore, it was investigated whether the deletion of *ompD* extended to *smvA*. This gene is adjacent to *ompD* in the *Salmonella typhimurium* chromosome and encodes a protein involved in the resistance to methyl viologen, a superoxide-generating agent. Although PCR failed to amplify the *smvA* gene from the *S. typhi* strain Ty2 genome, it was possible to amplify it from the chromosome of the clinical strains. On the other hand, hybridization analyses showed that the *smvA* gene is present in all the *S. typhi* strains tested. In contrast to the other *Salmonella* serovars, *S. typhi* strain Ty2 and the clinical isolates showed sensitivity to methyl viologen, suggesting that *smvA* gene is inactive in *S. typhi*. In conclusion, the *ompD–smvA* region is variable in structure among *Salmonella* serovars. It is hypothesized that the absence of *ompD* may suggest a role in host specificity.

Keywords: *Salmonella*, outer-membrane protein, *smvA*, genetic variability, host specificity

INTRODUCTION

*Salmonella typhi* causes the systemic disease typhoid fever only in its human host. The closely related *Salmonella typhimurium* causes a typhoid-like systemic disease in the mouse, but most frequently causes only a self-limited gastroenteritis in the human host. Several genetic variations between *S. typhi* and *S. typhimurium* have been suggested to contribute to their respective host specificity and infectivity. The relative order of genes on the *S. typhimurium* and *S. typhi* chromosomes is different, as is the relative stability of their genomes. In addition, the *S. typhi* genome is considered to be fluid because different chromosome structures are found among clinical isolates (Echeita & Usera, 1998; Liu & Sanderson, 1996). Many of these genomic rearrangements are thought to be due to homologous recombination events between pairs of the seven rRNA (rrn) operons. Thus, it has been postulated that genomic
rearrangements, rather than mutations, are responsible for producing the ribotype heterogeneity in S. typhi (Ng & Sanderson, 1999). In contrast, no rrn-mediated rearrangements have been detected among wild-type strains of S. typhimurium (Liu & Sanderson, 1995b; Liu & Sanderson, 1996) in spite of the high frequency of rearrangements observed for the S. typhimurium strain LT2 when grown in culture (Anderson & Roth, 1981). S. typhi appears to be exceptional among enteric bacteria because of its significant genome structure plasticity (Krawiec & Riley, 1990; Liu & Sanderson, 1996).

Because the sequences of conserved genes between S. typhi and S. typhimurium are nearly identical, they are said to be homologous (Zahrt & Maloy, 1997; Zahrt et al., 1994). However, it is unlikely that the minor fraction (1–2%) of nucleotide base pair differences between genes conserved in these two serovars contributes significantly to their remarkably different host specificities. Rather, the striking differences in S. typhi and S. typhimurium host specificity is most likely due to the gross rearrangements one observes when comparing their structures at a more macroscopic level. The substitutions and insertions between S. typhi and S. typhimurium genomes range in size from islands comprised of more than 100 genes to smaller substitutions corresponding to single ORFs (McClelland & Wilson, 1998). In addition, about 20% of the S. typhi genome is not present in S. typhimurium and vice versa (Lan & Reeves, 1996). Therefore, it is possible that as many as 900 S. typhi genes could be serovar-specific, a subset of which is likely to be involved in determining host range (Bäumler et al., 1998).

In this work, we describe one of the smaller interserovar differences in genome structure between S. typhi and S. typhimurium. The region of the S. typhimurium genome including smvA, ompD and yddG genes is different in S. typhi. These genes encode proteins that confer resistance to methyl viologen, to an abundant outer-membrane porin and to a putative transmembrane permease, respectively. The primary structure of the region containing these functions is genetically variable among Salmonella serovars.

**METHODS**

**Bacterial strains and growth conditions.** The Salmonella clinical strains used in this study are listed in Table 1. The S. typhi strains (STH) were obtained from the Infectious Diseases Hospital (Hospital Lucio Córdova) in Santiago, Chile. Bacteria were grown routinely at 37 °C in Luria–Bertani broth (LB; Bacto tryptone, 10 g l\(^{-1}\); Bacto yeast extract, 5 g l\(^{-1}\); NaCl, 5 g l\(^{-1}\)) and aerated by shaking. When required, LB was supplemented with ampicillin (100 mg l\(^{-1}\)) and tetracycline (10 mg l\(^{-1}\)). Media were solidified by the addition of agar (15 g l\(^{-1}\)).

**Genetic techniques.** Transductional crosses were carried out at low m.o.i. using the high-frequency transducing phage P22 HT105/1 int-201 as described by Maloy (1990). A recombinant S. typhi Ty2 ompD\(^{+}\) strain was obtained by transducing the wild-type ompD gene from S. typhimurium LT2 to S. typhi strain Ty2. In a first cross, the ompD::Tn10 allele was transduced using phage P22 from S. typhimurium strain MST2944 to S. typhi strain Ty2, selecting for tetracycline resistance. In a second cross, the wild-type ompD allele was transduced from S. typhimurium strain LT2 to S. typhi strain Ty2 ompD::Tn10 to produce the S. typhi strain Ty2 ompD\(^{+}\). The latter was obtained by selecting for loss of tetracycline resistance on Bochner plates (Maloy & Nunn, 1981). Serotypes of the transductants were confirmed by agglutination using specific antisera against O-antigen groups D1 and B.

**Preparation of outer-membrane proteins (OMPs).** Outer-membrane fractions were prepared as described by Lobos & Mora (1991), based on a modification of the method of Schnaitman (1971). Bacteria were grown overnight at 37 °C and harvested by centrifugation at 13000 g for 15 min. Cell pellets were washed and resuspended in 1 ml 10 mM Tris/HCl pH 8.0 buffer. Bacteria were disrupted by sonication (Vibra Cell, Sonics & Materials) for 100 s and centrifuged at 8000 g for 5 min. Supernatants were collected and centrifuged at 13000 g for 30 min. Supernatants were then discarded and pellets were resuspended in 500 µl buffer containing 10 mM Tris/HCl pH 8.0, 10 mM MgCl\(_2\), 2% Triton X-100. After incubation at 37 °C for 30 min, mixtures were centrifuged at 13000 g for 30 min. The insoluble outer-membrane fraction was resuspended in buffer consisting of 100 mM Tris/HCl pH 8.0, 2% SDS. SDS-PAGE was performed in 12.5% polyacrylamide slabs as described by Lobos & Mora (1991).

**PCR amplifications.** All amplifications were conducted using a Perkin Elmer thermal cycler (GeneAmp PCR System 2400) and Taq polymerase (Gibco-BRL). The final volume in the tubes for amplification was 50 µl and consisted of 1× Taq PCR buffer, 1.5 mM MgCl\(_2\), each dNTP at a concentration of 200 µM, 50 pmol of each primer, 0.1 µg DNA and 1.25 U Taq polymerase. Standard conditions for amplification were 30 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, followed by a final extension step at 72 °C for 5 min. Primers OMPD1 (5′-GAC AAA GAC AAA ACC CGT T-3′) and OMPD2 (5′-CGT CCA GCA GGT TGA TTT T-3′) were used to amplify a 740 bp internal fragment of the ompD gene (Singh et al., 1996). Primers SMVA1 (5′-CTT AAC CGC CGG CTA GTA TTT-3′) and SMVA2 (5′-GCT CAA CCA CAT CCC TAC C-3′) were designed from the reported smaA sequence (GenBank accession no. D26057) and used to amplify a 940 bp fragment internal to smaA.

**Southern hybridizations.** Plasmid pNK2883 (Kleckner et al., 1991) was cleaved with BamHI, and the 3 kb fragment with the tetRA genes was purified, labelled using the Bioprime DNA labelling system (Gibco-BRL) and used as the probe for Tn10. Hybridization probes for ompD and smaA were generated by PCR using a mixture of dNTPs containing biotin-14-dCTP and purified by using the Concert gel extraction system (Life Technologies). Genomic DNA of Sal- monella serovars was prepared as described by Sambrook et al. (1989), restricted with PstI (Gibco-BRL) and the fragments were separated on a 0.8% agarose gel. The DNA was then transferred onto a nylon membrane and cross-linked by UV irradiation. Hybridization was performed in solutions without formamide at 65 °C. Two 15 min washes were performed at 65 °C in a buffer consisting of 0.5 M Na\(_2\)HPO\(_4\), pH 7.2, 2% SDS, 1 mM EDTA. Hybridization was detected by using the nonradioactive Photogene nucleic acid detection system (Life Technologies) and XAR-5 Kodak films.
### Table 1. *Salmonella* strains used in this study

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*SARB, Salmonella Reference Collection B (Selander et al., 1996).† S. Maloy, University of Illinois, USA; ISP, Instituto de Salud Pública (Public Health Institute), Santiago, Chile.

**Tests for resistance to methyl viologen (paraquat).** Each of the *Salmonella* strains tested was isolated on MacConkey-lactose agar, and single colonies were streaked onto LB agar plates containing 0.5 mM methyl viologen, an agent that generates superoxide and oxygen radicals (Farr & Kogoma, 1991). Plates were incubated overnight at 37 °C and scored for bacterial growth. To determine the minimal dose of methyl viologen required to kill *Salmonella* strains, a lawn of the strain being tested was seeded on LB agar plates and 10 µl of serial dilutions of methyl viologen stock were added. Plates were incubated overnight at 37 °C and scored for bacterial growth.
RESULTS

*S. typhi* does not produce the OmpD porin

Previously, we have reported that *S. typhi* strain Ty2 does not produce the OmpD porin (Calderón et al., 1984). In contrast, OmpD is one of the most abundant proteins in the *S. typhimurium* outer membrane (Lee & Schnaitman, 1980; Nikaido & Vaara, 1985; Nikaido, 1996). To investigate the presence of OmpD among the majority of *Salmonella* serovars most frequently associated with systemic infection of vertebrate hosts, the OMPs of a variety of *Salmonella* serovars were analysed by SDS-PAGE. Electrophoretic profiles of OMPs produced by *Salmonella* enteritidis, *Salmonella* paratyphi A, *Salmonella* paratyphi B, *Salmonella* paratyphi C, *Salmonella* choleraesuis, *Salmonella* gallinarum, *Salmonella* pullorum, *Salmonella* dublin, *Salmonella* saintpaul, *Salmonella* muenchen, *Salmonella* heidelberg and *Salmonella* montevideo showed that all of these serovars produce a major protein migrating as *S. typhimurium* OmpD porin (Fig. 1a).

The same protein band was observed when this analysis was extended to include *Salmonella* agona, *Salmonella* anatum, *Salmonella* brandenburg, *Salmonella* decatur, *Salmonella* derby, *Salmonella* duisberg, *Salmonella* emek, *Salmonella* infantis, *Salmonella* miami, *Salmonella* newport, *Salmonella* panama, *Salmonella* reading, *Salmonella* rubislaw, *Salmonella* schwarzengrund, *Salmonella* sendai, *Salmonella* senftenberg, *Salmonella* stanley, *Salmonella* thompson, *Salmonella* typhicum and *Salmonella* wien (data not shown). This protein band disappeared from the SDS-PAGE OMP profiles when the *ompD*: *Tn*10 allele was transduced from *S. typhimurium* strain MST2944 to *S. enteritidis*, *S. paratyphi* A, *S. paratyphi* B, *S. pullorum* and *S. gallinarum* recipient strains, by using phage P22 (Fig. 1b). These data indicate that the protein band corresponds to the OmpD porin in these serovars, making it highly probable that it also represents OmpD in all the *Salmonella* serovars included in this study. On the other hand, the analysis of OMPs from *S. typhi* strain Ty2 (Fig. 1a) and 26 independent clinical isolates of *S. typhi* (data not shown) indicated that OmpD is not produced by these strains.

The finding that *S. typhi* strains do not produce OmpD can be explained by one of two alternative hypotheses. *S. typhi* may have an *ompD* gene that is not expressed, or there may be no homologous *ompD* gene in *S. typhi*. Because the current sequence data produced by the *S. typhi* Sequencing Group at the Sanger Center (ftp://ftp.sanger.ac.uk/pub/pathogens/st/ST.dbs) do not include an OmpD coding sequence, sequence data alone do not allow the distinction between these two hypotheses. Therefore, a combination of genetic and biochemical experiments was designed to determine whether the *ompD* gene is absent from the *S. typhi* genome.

PCR and DNA hybridization analyses indicate that the *ompD* gene is absent from the *S. typhi* genome

To determine whether the *ompD* gene is present in *S. typhi*, we first attempted to amplify a 740 bp fragment internal to *ompD* using PCR, with *ompD*-specific oligonucleotide primers (Singh et al., 1996). With these primers, no product was obtained when DNA isolated...
from *S. typhi* strain Ty2, or DNA isolated from each of 26 independent *S. typhi* clinical isolates, was used as template (see Fig. 2b for example). In contrast, a product of the predicted size was amplified using the same primers and DNA templates prepared from all the other *Salmonella* serovars tested (Fig. 2c).

Since deficient primer annealing due to low homology could account for the absence of PCR amplification of the *ompD* gene, the presence of the *ompD* gene in *S. typhi* was evaluated directly by DNA hybridization. This method is less dependent on the sequence homologies between the *S. typhi* and *S. typhimurium* serovars. Therefore, the 740 bp *ompD* fragment obtained from *S. typhimurium* was labelled and used to probe Southern blots of *S. typhi* DNA. The *ompD* probe hybridized neither with DNA obtained from *S. typhi* strain Ty2, nor with DNA from each of 10 randomly chosen *S. typhi* clinical isolates (Fig. 3a). In contrast, it did hybridize with a band of approximately 4.4 kb in DNA samples obtained from *S. typhimurium* strain LT2 (Fig. 3a). This small band indicates a DNA fragment containing the wild-type *ompD* gene. The DNAs of several *Salmonella* serovars were also examined by Southern blot hybridization using the highly specific probe to the *ompD* gene. Hybridization was observed in three patterns, one of which was identical to that observed using *S. typhimurium* DNA and included *S. brandenburg*, *S. emek*, *S. haifa*, *S. heidelberg*, *S. infantis*, *S. miami*, *S. panama*, *S. paratyphi A*, *S. pullorum*, *S. reading*, *S. schwarzengrund* and *S. wien* serovars. The second hybridization pattern revealed a band of approximately 14 kb in DNA samples obtained from *S. agona*, *S. anatum*, *S. choleraesuis*, *S. decatur*, *S. derby*, *S. dublin*, *S. duisberg*, *S. enteritidis*, *S. gallinarum*, *S. montevideo*, *S. muenchen*, *S. newport*, *S. paratyphi C*, *S. rubislaw*, *S. saintpaul*, *S. sendai*, *S. stanley*, *S. thompson* and *S. typhisuis* serovars. The third pattern of hybridization revealed a band of approximately 11.5 kb, and was only observed in DNA samples obtained from *S. paratyphi B* (Fig. 3b). These results strongly suggest that the *ompD* locus is widely distributed among *Salmonella* serovars, with the exception of *S. typhi*.

**The *S. typhimurium ompD* gene can be transduced to and expressed in *S. typhi***

To understand the genetic variation of the *ompD* gene between *S. typhi* and *S. typhimurium*, we assessed the feasibility of constructing a derivative of *S. typhi* harbouring the *S. typhimurium* *ompD* gene (*S. typhi* strain Ty2 *ompD*). Previously, it has been shown that multiple sequence differences in genes conserved between these two serovars present a formidable barrier to the construction of interspecific hybrids. This is because both mismatch repair and the RecBCD recombination pathways inhibit homologous recombination events (Zahrt & Maloy, 1997; Zahrt et al., 1994). Nonetheless, it is possible to introduce small regions of the *S.
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**Fig. 3.** Southern blot hybridization of genomic DNAs from *Salmonella* strains to an *ompD*-specific probe. DNA samples obtained from *S. typhi* clinical strains (a) or several *Salmonella* serovars (b) were digested with *Pst*I. The fragments were resolved by electrophoresis through 0.8% agarose gel and probed at high stringency with a PCR-amplified 740 bp fragment of *ompD*.

**Fig. 4.** Southern blot hybridization of genomic DNAs from interspecific hybrids between *S. typhimurium* and *S. typhi*. DNA samples were digested with *Pst*I, fragments were resolved by electrophoresis through 0.8% agarose gel, then probed at high stringency with a PCR-amplified 740 bp fragment of *ompD* (b) and with a DNA fragment internal to the *Tn10 tetAR* genes (a).

typhimurium* genome into *S. typhi* by generalized transduction with temperate phage P22 with low but measurable efficiencies. Therefore, we designed a two-step genetic approach using P22-mediated transduction to construct the *S. typhi* strain Ty2 *ompD*⁺.

In a first cross, we introduced an *ompD::Tn10* allele from donor *S. typhimurium* strain MST2944 into recipient *S. typhi* strain Ty2 by generalized transduction with phage P22 and selection for recombinants that had acquired resistance to tetracycline. PCR amplification of...
the ompD gene from *S. typhimurium* strain LT2 ompD::Tn10 and *S. typhi* strain Ty2 ompD::Tn10 DNA templates yielded the expected 740 bp product, revealing that the amplified fragment of ompD does not include the site of the *ompD*::Tn10 insertion (Fig. 2a). The presence of the *ompD*::Tn10 allele in the chromosome of these interspecific hybrids was confirmed by Southern hybridization, using a probe specific to the *tetRA* genes of Tn10. As shown in Fig. 4(a), the Tn10 probe hybridizes with a band of approximately 143 kb in *PstI*-restricted DNA samples obtained from *S. typhi* strain Ty2 ompD::Tn10 and *S. typhimurium* strains MST2944 ompD::Tn10 and LT2 ompD::Tn10. A comparison of the OMP profiles by SDS-PAGE clearly showed that *S. typhimurium* strain LT2 ompD::Tn10 and *S. typhi* strain Ty2 ompD::Tn10 do not express the OmpD porin (Fig. 5).

In a second cross, the *S. typhi* strain Ty2 ompD::Tn10 was used as the recipient of the *ompD*+ allele from *S. typhimurium* strain LT2, selecting for transductants that had lost the tetracycline resistance determinant (Maloy & Nunn, 1981). PCR amplification of genomic DNA isolated from the *S. typhi* strain Ty2 ompD+ using the *ompD*-specific primers yielded a 740 bp product (Fig. 2a). Accordingly, a band of approximately 44 kb was observed when *PstI*-digested DNA samples obtained from *S. typhi* Ty2 ompD+ were hybridized with the *ompD* probe (Fig. 4b). A band of the same size was also observed when *S. typhimurium* DNA was probed, revealing the presence of the *ompD*+ allele. Remarkably, we confirmed the presence of the OmpD porin in OMP preparations of the *S. typhi* strain Ty2 ompD+ by SDS-PAGE (Fig. 5). These results show that the *ompD* gene can be introduced into the *S. typhi* genome, and can be expressed in this genetic background, suggesting that there are no species-specific factors required for *ompD* expression in *S. typhi*.

### Absence of *ompD* in *S. typhi*

The observation that *S. typhi* strain Ty2, as well as the clinical strains, are missing the *ompD* gene is suggestive of a deletion in the *S. typhi* genome with respect to the *S. typhimurium* genome that may extend beyond the *ompD* gene. Analysis of the *S. typhimurium* chromosome reveals that *ompD* is adjacent to *smvA* (Hongo et al., 1994). It has been suggested that SmvA is an inner-membrane protein involved in the export of ammonium quaternary substrates, such as methyl viologen, by exporting them to the extracellular space (Hongo et al., 1994). Methyl viologen generates superoxide and oxygen radicals (Farr & Kogoma, 1991) and has been used *in vitro* to mimic the oxidative environment found within macrophages (Buchmeier et al., 1997).

Therefore, *S. typhi* strains, as well as other *Salmonella* serovars were tested for their sensitivity or resistance to methyl viologen following the same procedure used to identify the *S. typhimurium* *smvA* gene (Hongo et al., 1994). Among 35 *Salmonella* serovars tested, only *S. typhi* strain Ty2 was unable to grow on Luria agar plates supplemented with 0.5 mM methyl viologen. In addition, the 26 clinical isolates of *S. typhi* used in this study were also unable to grow in the presence of the same amount of methyl viologen. To determine the level of resistance to methyl viologen, some strains were tested for their ability to grow in the presence of a range of concentrations of this compound. The highest concentration that allowed the growth of *S. typhi* strain Ty2 and *S. typhimurium* strain LT2 were 0.4 mM and 50 mM, respectively. These results suggest either that *S. typhi* is missing the *smvA* gene, or that the *smvA* gene is not functional in this serovar.

To distinguish between these two alternatives, primers from the reported *smvA* sequence (GenBank accession no. D26057) were designed to amplify a portion of this gene. Amplification of a *S. typhi* strain Ty2 DNA template did not yield a product internal to *smvA*. In contrast, amplification of chromosomal DNA isolated from *S. typhimurium* strains LT2 ompD+ and LT2 ompD::Tn10, as well as *S. typhi* strains Ty2 ompD::Tn10 and Ty2 ompD+, resulted in the expected 940 bp product (Fig. 6a). Remarkably, DNA obtained from four randomly chosen clinical isolates of *S. typhi*, which were sensitive to methyl viologen, yielded an amplification product of the expected size (Fig. 6b). Thus, although the SmvA function is absent from all tested *S. typhi* strains, the *smvA* gene appears to be present in *S. typhi* clinical strains.

In addition, the 940 bp *smvA* fragment from *S. typhimurium* was labelled and used to probe Southern blots of *S. typhi* DNA digested with *PstI*. Since there is a single *PstI* site within the *S. typhimurium* *smvA* sequence reported, and this site is present in the probe, the *smvA* probe hybridized with two fragments (approx. 44 and 11.8 kb) present in *S. typhimurium* strain LT2 DNA.

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**Fig. 5.** SDS-PAGE analysis of porins from *S. typhimurium* and *S. typhi* interspecific hybrid strains. OMP preparations were obtained from bacteria grown aerobically at 37°C in LB broth to stationary phase, resolved by electrophoresis through 12.5% acrylamide and stained with Coomassie blue. Under the bacterial growth conditions used, OmpF porin is not detected in the OMP profiles.
demonstrate that, due to the absence of the S serovars, with the exception of in the outer membrane of many different

The OmpD porin is one of the most abundant proteins containing both ompD (Fig. 7). The smaller band represents a fragment carrying the smvA probe hybridized with a single band of approximately 14.5 kb in DNA samples obtained from this strain (Fig. 7). The same hybridization band was observed when DNA isolated from 10 randomly chosen clinical isolates of S typhi clinical strains does not have the PstI site present in the S typhimurium smvA gene (unpublished observation).

**DISCUSSION**

The OmpD porin is one of the most abundant proteins in the outer membrane of many different Salmonella serovars, with the exception of S typhi. Our results demonstrate that, due to the absence of the ompD gene, S typhi does not produce the OmpD porin. Both PCR and Southern analyses show that the OmpD coding sequence is absent from the S typhi genome. Nonetheless, it was possible to construct a derivative of S typhi carrying the S typhimurium ompD gene which expressed the porin. Presumably, this interspecific hybrid carries only a small cassette of genes that includes ompD, and provides us with a control suggesting that there are no species-specific factors required for ompD expression in S typhi.

To construct a recombinant strain with a single copy of ompD in the proper genetic position, we introduced an ompD::Tn10 allele from S typhimurium MST2944 donor strain into recipient S typhi strain Ty2 by generalized transduction with S typhimurium phage P22. This transduction was originally performed by Liu & Sanderson (1995a, b) as one of a systematic set of transductions used to assign genes on the S typhi strain Ty2 genome to positions corresponding to those on the S typhimurium genetic map. Using this approach, Liu

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*Fig. 6.* Amplification of the smvA gene from Salmonella strains. PCR amplifications were performed as described in Methods, using primers internal to smvA. Chromosomal DNA templates were obtained from (a) interspecific hybrids between S typhimurium and S typhi, and (b) S typhi clinical strains. RFLP analysis indicated that the smvA fragment amplified from S typhi clinical strains does not have the PstI site present in the S typhimurium smvA gene (unpublished observation).

*Fig. 7.* Southern blot hybridization of genomic DNAs from Salmonella strains to a smvA-specific probe. DNA samples were digested with PstI. The fragments were resolved by electrophoresis through 0.8% agarose gel and probed at high stringency with a PCR-amplified 940 bp fragment of smvA. Only one band was observed when DNA isolated from S typhi was probed because S typhi smvA does not have the PstI site present in the S typhimurium smvA gene.
& Sanderson (1995a, b) concluded that the ompD gene is located in the centre of a 500 kb inversion which includes the replication terminus of the \textit{S. typhi} strain Ty2 chromosome. The \textit{ompD}: \textit{Tn10} allele from \textit{S. typhimurium} can be transduced into the \textit{S. typhi} genome even though the recipient does not have the corresponding homeologous gene. This is because P22 packages about 44 kb of DNA, and P22-mediated generalized transduction permits homologous recombination events to occur in genes flanking a region of DNA that is not present in the recipient genome.

Our Southern blot analyses show that all \textit{S. typhi} strains tested retain an \textit{smuA} coding sequence, but they are nevertheless sensitive to methyl viologen, suggesting that the gene is not expressed in these strains. Alternatively, they may be lacking or be defective in additional genes required for methyl viologen resistance. In \textit{Escherichia coli}, a related Gram-negative bacillus, at least three genes dispersed on the bacterial genome are required for methyl viologen resistance, and mutations in each of these genes confer sensitivity to methyl viologen (Morymio, 1988; Morymio et al., 1992). Transductants of \textit{S. typhi} strain Ty2 carrying the \textit{ompD} allele and the cotransduced \textit{smuA} gene express the OmpD porin in the outer membrane, and acquire resistance to methyl viologen (unpublished observation), suggesting that the \textit{smuA} gene in clinical isolates of \textit{S. typhi} may not be functional. Thus, the \textit{ompD}–\textit{smuA} region is genetically variable among different \textit{Salmonella} serovars.

The \textit{ompD} and \textit{smuA} genes have been mapped at centisomes 33-7 and 38-6, respectively, in the \textit{S. typhimurium} chromosome (Sanderson et al., 1995). Because both genes are cotransducible with phage P22, they cannot be that far apart. Instead, our results confirm those of Singh et al. (1996) that place three \textit{S. typhimurium} genes, \textit{ompD}, \textit{smuA} and \textit{narU}, at centisome 33-7 (GenBank accession no. D26057). Analysis of the \textit{S. typhi} genome sequence data released by the Sanger Center (http://www.sanger.ac.uk/Projects/S_typhi/blast_server.shtml and ftp://ftp.sanger.ac.uk/pub/pathogens/st/ST.db) reveals that \textit{S. typhi} has two \textit{nar} operons, 85\% and 81\% identical at the nucleotide level to the \textit{E. coli} \textit{narGHIJ} and \textit{narZ} operons, respectively. Both \textit{nar} operons are also present in \textit{S. typhimurium} (Barrett & Riggs, 1982; Spector et al., 1999), but only one of them (\textit{narGHIJ}) has been mapped, lying at centisome 38-6 (Casse et al., 1973; Sanderson et al., 1995). In \textit{E. coli}, the \textit{narU} gene is located immediately upstream of the \textit{narZ} operon (Gennis & Stewart, 1996). Given the remarkable conservation of gene order between \textit{E. coli} strain K12 and \textit{S. typhimurium} strain LT2 (Krawiec & Riley, 1990), we can assign the \textit{narZ} operon to a map position at centisome 33-7 on the \textit{S. typhimurium} genome, adjacent to the \textit{ompD}–\textit{smuA}–\textit{narU} region. Recently, the Genome Sequence Center from the Washington University School of Medicine (http://genome.wustl.edu/gsc/Projects/bacterial/salmonella.shtml) sequenced an ORF upstream of the \textit{S. typhimurium} \textit{ompD} gene. This ORF, designated \textit{yddG}, encodes a putative transmembrane permease. Notably, \textit{yddG} is not present in the \textit{S. typhi} \textit{ompD} sequence data released by the Sanger Center, suggesting that the genetic variability in the \textit{ompD}–\textit{smuA} region of the \textit{Salmonella} genome also includes the \textit{yddG} locus.

Because \textit{S. typhi} is the only \textit{Salmonella} serovar missing the \textit{ompD} gene, the determination of its presence in other \textit{Salmonella} serovars has a clinical significance. This kind of assay allows us to distinguish \textit{S. typhi} from \textit{S. paratyphi} serovars that cause systemic human infections presenting nearly identical symptoms. Thus, a PCR assay could be developed by using primers hybridizing upstream and downstream of the presumed deletion in the \textit{S. typhi} strains, where the length of the amplified segment should discriminate between the different \textit{Salmonella} serovars responsible for clusters of typhoid-like fevers and thus allow a more accurate assessment of their epidemiology.

What is the role of the \textit{ompD} gene in \textit{Salmonella} infection? The \textit{ompD} gene does not appear to play a major role in the virulence of \textit{Salmonella} serovars, because the effects of mutations in the \textit{ompD} gene on the virulence of \textit{S. typhimurium} are subtle. Dorman et al. (1989) found that a strain with a \textit{Tn10} insertion in \textit{ompD} has a slightly greater LD\textsubscript{50} than its otherwise isogenic wild-type parent. However, Meyer et al. (1998) found that there was no statistically significant difference between the LD\textsubscript{50} of \textit{S. typhimurium} wild-type and \textit{ompD} mutant strains.

Although it seems likely that the \textit{ompD} gene plays only a minor role in virulence, it may be important for determining host specificity. Many studies have characterized the genetic determinants of \textit{Salmonella} virulence; however, very little is known about the genetic determinants of host specificity. Initial attempts to define the genetic basis of host specificity revealed that its determinants in \textit{Salmonella} are likely to be multifactorial. In generalized transduction experiments with phage P22 grown in a \textit{S. typhimurium} donor and with \textit{S. typhi} recipients, no virulent recombinants that could kill mice were obtained (Zahrt, 1998). These negative results indicate that multiple genetic differences between these serovars must contribute to host specificity.

We hypothesize that the \textit{ompD}–\textit{smuA} region contributes to host specificity, because the presence of \textit{ompD} is strongly correlated with the ability of \textit{Salmonella} serovars to grow in alternative, non-human hosts. \textit{OmpD} is present in all \textit{Salmonella} serovars, such as \textit{S. typhimurium} and \textit{S. enteritidis}, that grow in multiple mammalian hosts. In contrast, \textit{OmpD} is absent from \textit{S. typhi}, which can grow only in a human host. Although the \textit{S. paratyphi} A, B and C serovars express OmpD, and were once thought to cause systemic infections only in humans, several reports have shown that \textit{S. paratyphi} serovars B and C are not restricted to a human host and can infect cattle and poultry (George et al., 1972; Thomas, 1978; Ojeniyi, 1984). The almost perfect correlation between the absence of \textit{ompD} and a host range restricted to humans raises the important question.
of whether S. paratyphi A also has an alternative vertebrate host.

In recent years, it has become clear that the outer-membrane porins are involved in the interactions of Gram-negative species, including Helicobacter pylori (Tufano et al., 1994), Neisseria gonorrhoeae (Bauer et al., 1999), Shigella flexneri (Bernardini et al., 1993), S. typhi (Blanco et al., 1997) and possibly Pasteurella haemolytica (Davies et al., 1997), with their specific hosts. The OmpD porin must play a critical role in the survival of S. typhimurium in certain environments, because OmpD facilitates the entry of some critical nutrients (Nikaido, 1996). Furthermore, OmpD may also play a critical role in the interactions between S. typhimurium and host cells, and is likely to be involved in the adhesion of S. typhimurium to murine macrophages (Nemg & Pistole, 1998).

The characterization of this important genomic difference between S. typhi and other Salmonella serovars that includes ompD is significant for the following three reasons. First, the report of similar small differences in genome structure among different serovars will enable the development of additional PCR assays for a more robust assessment of the epidemiology of Salmonella outbreaks. Second, by demonstrating that generalized transduction can be used to construct interspecific hybrids between different Salmonella serovars, it is possible to take a genetic approach to assessment of the contributions of small genomic differences between two serovars to differences in host specificity. Third, the correlation between the presence of the ompD gene and the ability of Salmonella serovars to infect vertebrate, non-human hosts is the first reported correlation between an individual gene and the host range of Salmonella serovars.

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