Differentiation of *Salmonella* Phase 1 Flagellar Antigen Types by Restriction of the Amplified *fliC* Gene

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The large antigenic diversity (over 2,300 serotypes) expressed by *Salmonella* strains can probably be observed at the genetic level. The phase 1 flagellin gene *fliC* was amplified, and the amplified fragment was cleaved with a mixture of both endonucleases *Taq I* and *Sca I*. The restriction patterns observed allowed differentiation of flagellar types b, i, d, j, l,v, and z. Flagellar group g (g,m, g,p, or g,m,s) could be differentiated from the other flagellar types. Flagellar types r and e,h could not be separated, although they could be distinguished from the other flagellar types studied. Practical applications of flagellar gene restriction are the distinction between serotype Gallinarum-Pullorum, which carries a cryptic gene for flagellar type g,m, and nonmotile Vi-negative variants of serotype Typhi, and the tentative assignment of nonmotile variants of *Salmonella* serotypes to a flagellar type.

There are presently 2,324 different *Salmonella* serotypes (5). In practice, only a limited set is really prevalent. For example, 15 serotypes made up 83.2% of the 12,726 clinical isolates studied at the French National *Salmonella* Center in 1991 (2). Serotypes Enteritidis and Typhimurium alone represent 71.9% of the clinical *Salmonella* isolates.

Although serotyping offers a very precise and reliable method for differentiating *Salmonella* strains, identification of the complete set of serotypes is a time-consuming process and requires the use of 167 specific serum samples. With the expanding availability of molecular biology methods, it is tempting to unveil the antigenic diversity where it is coded for, i.e., on the DNA. Most *Salmonella* strains possess two structural genes (*fliC* and *fliB*) coding for flagellins. No more than one of these structural genes is expressed at a time in one bacterial cell. Nonmotile strains generally possess the structural genes but are unable to build up a functional flagellum (4). Early genetic studies have shown the nonmotile serotype Gallinarum-Pullorum to have a structural gene for type gm flagellin (12). Several sequences of the gene coding for phase 1 flagellin (*fliC*) have been published (3, 7–11), and some are under study in our laboratory. The remarkable feature of *fliC* alleles is the sequence conservation of distal parts of the gene, thus making the gene of any serotype suitable for easy amplification.

The purpose of the present study was to differentiate the phase 1 flagellar genes of the major serotypes by a simple method, restriction of the amplified *fliC* gene.

**MATERIALS AND METHODS**

**Bacterial strains.** The strains used in the present study are listed in Table 1. FNSC strains were from the French National *Salmonella* Center (located in our laboratory). All other strains were from the Collaborating Center for Reference and Research on *Salmonella*, World Health Organization (also located in our laboratory).

**DNA extraction.** Bacterial DNA was prepared as described by Brenner et al. (1).

**Primers used for amplification.** For the amplification of the phase 1 flagellin gene, the following two primers were used (10): AAG GAA AAG ATC ATG GCA (primer 1) and TTA ACG CAG TAA AGA GAG (primer 2).

**Amplification of the *fliC* gene.** DNA was amplified by the polymerase chain reaction (PCR) (6). A reaction mixture of 100 µl contained 1 µl (1 to 10 µg) of extracted DNA, 50 to 100 pM (each) primer (primers 1 and 2), 200 µM nucleotide mixture (ultrapyrimidine triphosphate set; Pharmacia Biotechnology LKB, Uppsala, Sweden), 10 µl of 10× Taq polymerase buffer (Amersham International, Amersham, United Kingdom), and 2.5 µl of Taq polymerase (Amersham). The reaction mixture was covered with 75 µl of mineral oil (Sigma Chemical Co., St. Louis, Mo.), and the PCR was performed with a Perkin-Elmer Cetus DNA Ther-

**TABLE 1.** Strains of *Salmonella* included in the present study

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Strain no.</th>
<th>Phase 1 flagellar antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhi</td>
<td>Ty2</td>
<td>d</td>
</tr>
<tr>
<td>Typhi</td>
<td>E 75 2099</td>
<td>d</td>
</tr>
<tr>
<td>Typhi</td>
<td>E 80 2027</td>
<td>j</td>
</tr>
<tr>
<td>Typhi</td>
<td>E 285K</td>
<td>j</td>
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<td>g,m</td>
</tr>
<tr>
<td>Dublin</td>
<td>65K</td>
<td>g,p</td>
</tr>
<tr>
<td>Montevideo</td>
<td>46K</td>
<td>g,m,s</td>
</tr>
<tr>
<td>Panama</td>
<td>73K</td>
<td>l,v</td>
</tr>
<tr>
<td>Brandenburg</td>
<td>24K</td>
<td>l,v</td>
</tr>
<tr>
<td>Bredeney</td>
<td>30K</td>
<td>l,v</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>LT2</td>
<td>i</td>
</tr>
<tr>
<td>Paratyphi B</td>
<td>8006</td>
<td>b</td>
</tr>
<tr>
<td>Hadar</td>
<td>473K</td>
<td>z10</td>
</tr>
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<td>Heidelberg</td>
<td>16K</td>
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<td>r</td>
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<td>Bovismoribicans</td>
<td>53K</td>
<td>r</td>
</tr>
<tr>
<td>Infantis</td>
<td>158K</td>
<td>r</td>
</tr>
<tr>
<td>Newport</td>
<td>12302</td>
<td>eh</td>
</tr>
<tr>
<td>Gallinarum-Pullorum</td>
<td>FNSC 4.83</td>
<td>a —*</td>
</tr>
<tr>
<td>Gallinarum-Pullorum</td>
<td>FNSC 51.66</td>
<td>a —*</td>
</tr>
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<td>Nonmotile O9,12</td>
<td>FNSC 10.90</td>
<td>a —*</td>
</tr>
<tr>
<td>Nonmotile O9,12</td>
<td>FNSC 14.90</td>
<td>a —*</td>
</tr>
<tr>
<td>Nonmotile O3,10</td>
<td>FNSC 5.89</td>
<td>a —*</td>
</tr>
</tbody>
</table>

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a —*, no flagellar antigen.
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The amplified and purified fltC gene was cleaved with different restriction enzymes according to the manufacturers' instructions. These enzymes were PstI (Amersham), EcoRII (GIBCO BRL Life Technologies, Renfrewshire, United Kingdom), HpaI (Pharmacia), HincII (Amersham), TaqI (New England Biolabs, Beverly, Mass.), and ScaI (Pharmacia).

Restriction fragments were separated by electrophoresis on a 2% agarose gel.

RESULTS AND DISCUSSION

Restriction profile of the phase 1 flagellin gene (fltC). The phase 1 flagellin gene of the 23 Salmonella strains studied could be amplified with primers 1 and 2. The size of the PCR product was 1.24 kb for serotype Typhi expressing phase 1 flagellar antigen j (H1-j) and 1.5 kb for all other strains (Fig. 1).
Restriction profile of the fliC gene from nonmotile strains. Both strains of serotype Gallinarum-Pullorum yielded a fliC restriction profile identical to those of the g,m and g,p types. This is compatible with the knowledge that serotype Gallinarum-Pullorum has a cryptic fliC gene coding for phase 1 antigen g,m (I2).

Three nonmotile Salmonella strains of unknown serotype (isolates 14.90, 10.90, and 5.89) were examined by amplification and restriction of fliC. The restriction patterns obtained were directly compared with the profiles of serotype Typhi (H1-d) and serotype Panama (H1-l,v) on an agarose gel (Fig. 3), which permitted identification of strain 14.90 as a nonmotile variant of a serotype possessing the fliC gene encoding phase 1 flagellin type l,v. The phase 1 flagellin gene of strain 10.90 could be determined as type d. These results were consistent with those obtained by ribotyping (1a), which identified strain 14.90 as serotype Panama and strain 10.90 as serotype Typhi. The restriction pattern that was obtained for the third strain (strain 5.89) could not be identified with any of the serotypes included in the present study.

The use of restriction patterns of fliC genes proved to be a useful method for facilitating the identification of strains that have lost their flagellar antigens but that have a nonexpressed fliC gene. The first important application of the method was the distinction of serotype Gallinarum-Pullorum from nonmotile, Vi-negative variants of serotype Typhi. Although the two serotypes have different habitats (serotype Gallinarum-Pullorum strictly infects poultry, whereas serotype Typhi is adapted to humans), they can be found in the environment or in contaminated food. The present biochemical methods cannot distinguish between serotype Gallinarum-Pullorum and nonmotile, Vi-negative strains of serotype Typhi.

To establish the restriction patterns of fliC genes as a tool for identifying the major phase 1 flagellar antigens on a genetic basis, it is necessary to examine the restriction profiles of more strains with a complete or incomplete serotype. The validation of the amplification-restriction approach will require a few years of work to ensure that no rare flagellar types mimic commonly encountered flagellar types. It is too early in the development process to compare the relative costs of serotyping and amplification-restriction. The material is obviously more expensive on the amplification-restriction side, but the labor costs can be reduced by automation.

We can foresee with excitement the day when the major Salmonella serotypes are identified by restriction of amplified genes which code for flagellar antigens and those which are associated with the synthesis of somatic antigenic factors.

ACKNOWLEDGMENTS

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REFERENCES


1a. Grimont, F. Personal communication.


