The Biological Base of Salmonella Phage Typing

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Introduction: The complexity of Salmonella transmission routes and the observation that Salmonella serovars diversify during evolution have led to the development of special methods for strain discrimination. Phage typing systems of Salmonella have been developed since the 30s (Marcuse, 1934), and still they are very helpful tools for epidemiological work (Rabsch, 1996). Nevertheless, their biological basis is not well understood. We have studied the Anderson typing system (1959) which has a long tradition, to answer the following questions:
- To which extent do host controlled modification/restriction systems interfere?
- What is the significance of different phage receptor sites for typing?
- How important are prophage controlled super-infection exclusion systems?

1. Host controlled modification and restriction of typing phages (Table 1)
The DNAs of the typing phages A1 and A7 share the same EcoRI pattern (Schmieger, 1999). We suggested that their different plating efficiencies on various hosts, which are the basis of the phage typing system, are caused by host controlled modification and restriction. When phage A1 was propagated on host strain BA1 [=1(BA1)], it was significantly restricted by the strain BA7. When surviving phages A1 (BA1) were propagated on BA7, the plating efficiency of the resulting lysate A1 (BA1, BA7) was nearly the same on both strains. Therefore, strain BA7 seems to express at least one host controlled modification/restriction system that is absent in strain BA1. BA1 has no such system, or it has a system which is (in addition to others) also expressed in BA7. Repropagation of the phages A1 (BA1, A7) in BA1, yielding lysate A1 (BA1, BA7, BA1), renders A1 sensitive to BA7-restriction.
Table 1: Efficiency of plating of Anderson phage A1 after propagation of different S. Typhimurium strains

<table>
<thead>
<tr>
<th>phages</th>
<th>strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BA1</td>
</tr>
<tr>
<td>A1 (BA1)</td>
<td>$4.5 \times 10^{10}$</td>
</tr>
<tr>
<td>A1 (BA1, BA7)</td>
<td>$1.5 \times 10^{9}$</td>
</tr>
<tr>
<td>A1 (BA1, BA7, BA1)</td>
<td>$3 \times 10^{10}$</td>
</tr>
</tbody>
</table>

2. Adsorption properties of various typing phages and the dependence on FhuA and TonB proteins (Table 2)
The bacterial receptor of the typing phages A8 and A18 (the latter being identical with phage ES18) is the FhuA protein which is responsible for ferrichrome uptake (Lucey and Nielands 1976). Another bacterial protein necessary for effective infection by these phages is the TonB-protein. The phages A1 and P22, on the other hand, use the O12 polysaccharid antigen as a receptor. Therefore, S. Typhimurium strains carrying fhuA and tonB mutations appear resistant against A8 and A18, but sensitive against A1 and P22 (Table 2).

Table 2: Influence of FhuA and TonB to the plating efficiency of Anderson typing phages

<table>
<thead>
<tr>
<th>strains</th>
<th>relevant markers</th>
<th>Anderson phages</th>
<th>phages</th>
<th>source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. fhu mutants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WR1173 (BA36)</td>
<td>fhuA::MudJ</td>
<td>+</td>
<td>-</td>
<td>this paper</td>
</tr>
<tr>
<td>SL1027/23 (SL1027)</td>
<td>fhuA</td>
<td>+</td>
<td>-</td>
<td>K. Hantke</td>
</tr>
<tr>
<td>WR1179 (BA36)</td>
<td>fhuB::MudJ</td>
<td>+</td>
<td>+</td>
<td>this paper</td>
</tr>
<tr>
<td>2. tonB mutants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR1001 (enb-7)</td>
<td>tonB</td>
<td>+</td>
<td>-</td>
<td>W. Rabsch</td>
</tr>
<tr>
<td>AIR36</td>
<td>tonB::MudJ</td>
<td>+</td>
<td>-</td>
<td>R. Tsolis</td>
</tr>
<tr>
<td>control strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA36</td>
<td></td>
<td>+</td>
<td>+</td>
<td>L.R. Ward</td>
</tr>
<tr>
<td>(Anderson</td>
<td>DT36</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>typing system)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL1027</td>
<td></td>
<td>+</td>
<td>+</td>
<td>B.A.D. Stocker</td>
</tr>
<tr>
<td>enb-7</td>
<td>trp, met</td>
<td>+</td>
<td>+</td>
<td>J.B. Neilands</td>
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<tr>
<td></td>
<td>ent- class II</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

3. Influence of superinfection exclusion systems (Table 3)
Most Anderson typing phages belong to the P22 like phage family (Schmieder 1999). Phage P22 has 2 different superinfection exclusion systems, sieA and sieB. Such protection systems may be present also in natural strains, because it has been shown that almost all natural isolates of Salmonella carry prophages, most of
which belong to the P22-group. In order to study the influence of the superinfection exclusion systems on typing phages, strain BA36 was lysogenized with P22 wildtype (both sie systems are active) and with a P22 mutant defective in both sie-systems. BA36 and its lysogenic derivatives were assayed with the Anderson typing phages. As Table 3 shows, most typing phages (grey) were excluded by at least one of the sie-systems of P22 wildtype (line 2), because the phages were inactive on BA36 (P22), but could effectively plate on the sie-defective mutant (line 3). Phages A12, A13 and 17 were suppressed on both lysogenic strains, indicating that they are homo-immune to phage P22.

Table 3: Influence of the P22 superinfection exclusion systems (sie) on Anderson typing phages

<table>
<thead>
<tr>
<th>Salmonella indicator strain</th>
<th>Anderson typing phages (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA36</td>
<td>+</td>
</tr>
<tr>
<td>BA36 (P22)</td>
<td>-</td>
</tr>
<tr>
<td>BA36 (P22 sieA, sieB)</td>
<td>+</td>
</tr>
</tbody>
</table>

References