Host Specificity of Salmonella typhimurium
Deoxyribonucleic Acid Restriction and Modification

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The restriction and modification genes of Salmonella typhimurium which lie near the thr locus were transferred to a restriction mutant of Escherichia coli. These genes were found to be allelic to the E. coli K, B, and A restriction and modification genes. E. coli recombinants with the restriction and modification host specificity of S. typhimurium restricted phage $\lambda$ that had been modified by each of the seven known host specificities of E. coli at efficiency of plating levels of about $10^{-4}$. Phage $\lambda$ modified with the S. typhimurium host specificity was restricted by six of the seven E. coli host specificities but not by the RII (fi$^-$ R-factor controlled) host specificity. It is proposed that the restriction and modification enzymes of this S. typhimurium host specificity have two substrates, one of which is a substrate for the RII host specificity enzymes.

Seven different restriction and modification host specificities of Escherichia coli have been described in the past few years (3-6, 19). The host specificity of an organism can be defined by the substrate, i.e., the sequence of nucleotide base pairs, recognized by the restriction endonuclease and modification methylase. Three host specificities (K, B, and A) are controlled by alleles that are located near serB (3, 7). The other host specificities are controlled by extrachromosomal elements. The two types of R factors (fi$^+$ and fi$^-$) control different host specificities (4, 5, 19), and the phage P1 and P1-like defective plasmid have alleles controlling different host specificities (3). Salmonella typhimurium has been reported to carry two different host specificities, the genes for which are near proC and thr (9, 10).

We have transferred the host specificity locus of S. typhimurium that lies near thr to E. coli by conjugation. These strains presented the opportunity to characterize this host specificity relative to the seven known host specificities of E. coli.

MATERIALS AND METHODS
Organisms. The S. typhimurium Hfr was obtained from K. Sanderson (18). The E. coli strains with A, P1, and 15 host specificities were obtained from W. Arber. The parental strain (HB129, Ara-Str$^\beta$) used for the construction of strains carrying the K, B, RI, and RII host specificities was described previously (17). A $\lambda$vir (C$\alpha$, b$\beta$, V$\gamma$, V$\delta$) phage was used for efficiency of plating (EOP) experiments. Phage stocks were prepared from plate lysates.

Media and buffers. Growth media and storage buffers were described previously (8).

Conjugation. Log-phase broth cultures were mixed at an Hfr to F$^-$ ratio of 0.1 at a final density of $5 \times 10^8$/ml. The mixed cultures were incubated at 37°C for 1 hr without agitation.

Efficiency of plating. Conditions for the EOP experiments were described previously.

Nomenclature. Host specificity nomenclature is that recommended by Arber and Linn (2).

RESULTS
Preparation of an E. coli recombinant with the hspS locus of S. typhimurium. An $r_B$-m$\alpha$ derivative of the HB 129 strain was obtained by mutagenesis with nitrosoguanidine (1). It was used as a recipient in a cross with a culture of S. typhimurium Hfr A (SR305) which was His$^+$ (hisD23) Stra$. Ara$^+$ Stra$^+$ His$^+$ clones were selected and recovered at about 0.001% of the input Hfr. Sixteen clones were purified and tested for their ability to restrict unmodified Avir stocks. Six of these recombinant clones restricted $\lambda$-O and $\lambda$-B phage at EOP of about $10^{-2}$. The remainder had an $r_B$-m$\alpha$ phenotype. Phage stocks prepared on the six $r_B$ recombinants were restricted (EOP of
10^-4) by the parental r_n^+ m_n^+ E. coli strain but were not restricted when plated on the recombinant r^+ strain. We therefore concluded that the r^+ clones were generated through recombination with the hsp locus of S. typhimurium and designate this phenotype as r_n^+ m_n^+. Since none of the r_n^+ recombinants had an m_n^+ phenotype, we also tentatively conclude that the hspB hspK hspA and hspS loci are allelic.

The host specificity range of hssS. Phage stocks of λ were prepared on strains of E. coli with the following host specificities: K, B, A, 15, P1, RI, RII, S, and r_n^- m_n^- Each of the modified λ phage was restricted at an EOP of about 10^-2 except for λ-S (Table 1). The λ-S stock was restricted by all the known E. coli host specificities except for RII, which on the average yielded λ-S plaques at an EOP of about 0.5 (Table 2). These data indicate that the RII host specificity does not restrict λ-S, but the S host specificity can restrict λ-R.

An F lac derivative of the r_n^+ m_n^- E. coli strain was constructed to determine if the hspS host specificity modified or restricted the phage fd. It did not.

**DISCUSSION**

The results of the intergeneric cross described above suggest that the hsp loci of S. typhimurium and E. coli are allelic. There are three known alleles of the hsp locus of E. coli (hspB, hspK, hspA), and the hspS locus represents a fourth allele. The hsp loci, hspB and hspK, are composed of three cistrons which contain information for the restriction endonuclease and modification methylase (8, 12, 13). The current hypothesis postulates that one cistron (hss) is responsible for host specificity, i.e., recognition of the substrate, and the other two cistrons (hsm and hsr) confer catalytic properties to the enzymes (6). The methylase appears to be composed of two different subunits (products of hss and hsm genes) and serves as a "core" protein for the endonuclease that is constructed by the addition of the third subunit (hsr gene) (14).

The subunits of the K and B endonucleases and methylases are interchangeable in vivo (8), and the purified K and B endonucleases are quite similar in their enzymatic and physical properties (15–17). However, complementation between the K and A hsp alleles has not been observed (3). It has been previously proposed that the allelic nature of the K and B hsp loci reflects an evolutionary relationship with the generation of new host specificities through alterations of the hss cistrons (6, 8). This would suggest that the hspA and hspS enzymes would also have properties similar to the hspK and hspB enzymes. The extent of evolutionary divergence between the particular hsp alleles could affect the interchangeability of the subunit structure (11). One can imagine then that, in the generation of new host specificities through changes of the hss cistron, some changes of the hsr and hsm cistrons would be necessary. This might explain the failure to find complementation between some hsp alleles, e.g., K and A, but not others, e.g., K and B. We propose that the K, B, A, and S host specificities represent a family of related restriction and modification enzymes, sharing physical and genetic properties which primarily differ in the hss cistron.

The host specificity controlled by the hspS locus is different from the B, K, A, 15, P1, and RI host specificities. The partial overlap of the RII and S host specificities is the first observation of its kind. One interpretation of this result is that the S host specificity involves two related sequences of nucleotide base pairs and one of these defines the RII host specificity. Thus the RII host specificity cannot restrict the S-modified DNA, but RII-modified DNA is restricted by the S host specificity. It is also possible that two hsp loci were introduced when the E. coli hspS strain was constructed. We have not isolated restriction mutants of this

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**Table 1. Efficiency of plating of phage λ on Escherichia coli "S"**

<table>
<thead>
<tr>
<th>Phage</th>
<th>E. coli &quot;S&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ-S</td>
<td>1.0</td>
</tr>
<tr>
<td>λ-O</td>
<td>1 × 10^-2</td>
</tr>
<tr>
<td>λ-B</td>
<td>3 × 10^-3</td>
</tr>
<tr>
<td>λ-K</td>
<td>7 × 10^-3</td>
</tr>
<tr>
<td>λ-A</td>
<td>1 × 10^-2</td>
</tr>
<tr>
<td>λ-15</td>
<td>1 × 10^-2</td>
</tr>
<tr>
<td>λ-P1</td>
<td>2 × 10^-2</td>
</tr>
<tr>
<td>λ-RI</td>
<td>3 × 10^-3</td>
</tr>
<tr>
<td>λ-RII</td>
<td>5 × 10^-3</td>
</tr>
</tbody>
</table>

**Table 2. Efficiency of plating of λ-S on Escherichia coli**

<table>
<thead>
<tr>
<th>Host</th>
<th>λ-O</th>
<th>λ-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli &quot;S&quot;</td>
<td>1 × 10^-3</td>
<td>1.0</td>
</tr>
<tr>
<td>E. coli B</td>
<td>5 × 10^-4</td>
<td>3 × 10^-3</td>
</tr>
<tr>
<td>E. coli K</td>
<td>5 × 10^-4</td>
<td>7 × 10^-4</td>
</tr>
<tr>
<td>E. coli A</td>
<td>2 × 10^-3</td>
<td>3 × 10^-3</td>
</tr>
<tr>
<td>E. coli 15</td>
<td>5 × 10^-4</td>
<td>1 × 10^-2</td>
</tr>
<tr>
<td>E. coli P1</td>
<td>3 × 10^-2</td>
<td>2 × 10^-2</td>
</tr>
<tr>
<td>E. coli RI</td>
<td>1 × 10^-5</td>
<td>5 × 10^-5</td>
</tr>
<tr>
<td>E. coli RII</td>
<td>5 × 10^-5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
strain which would eliminate one of the above explanations.

Since 5-methyl-cytosine is the basis of the RII modification (R. Yoshimori, D. Roulland-Dussoix, H. M. Goodman, and H. W. Boyer, unpublished data), it will be of interest to determine the methylated nucleotide(s) of the S host specificity modification enzyme.

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LITERATURE CITED