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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 restrictionless 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 λ that had been modified by each of the seven known host specificities of *E. coli* at efficiency of plating levels of about 10^{-2}. Phage λ 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^R) used for the construction of strains carrying the K, B, RI, and RII host specificities was described previously (17). A λvir (C_1^-, b_2, V_2, V_1V_3) 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 5×10^8/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_B^+ 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*) Str^S. Ara⁺ Str^R 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 λvir stocks. Six of these recombined clones restricted λ·O and λ·B phage at EOP of about 10^{-2}. The remainder had an r_B^- m_B^+ phenotype. Phage stocks prepared on the six r⁺ recombinants were restricted (EOP of

10^{-4}) by the parental $r_B^+ m_B^+$ *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 $r_S^+ m_S^+$. Since none of the r_S^+ recombinants had an m_B^+ 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 $r_B^- m_B^-$. 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 $r_S^+ m_S^+$ *E. coli* strain was constructed to determine if the *hspS* host specificity modified or restricted the phage fd. It did not.

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

Phage	*E. coli* "S"
λ·S	1.0
λ·O	1×10^{-2}
λ·B	3×10^{-3}
λ·K	7×10^{-3}
λ·A	1×10^{-2}
λ·15	1×10^{-2}
λ·P1	2×10^{-2}
λ·RI	3×10^{-3}
λ·RII	5×10^{-3}

TABLE 2. *Efficiency of plating of λ·S on Escherichia coli*

Host	λ·O	λ·S
E. coli "S"	1×10^{-2}	1.0
E. coli B	5×10^{-4}	3×10^{-3}
E. coli K	5×10^{-4}	7×10^{-4}
E. coli A	2×10^{-3}	3×10^{-2}
E. coli 15	5×10^{-4}	1×10^{-3}
E. coli P1	3×10^{-3}	2×10^{-2}
E. coli RI	1×10^{-5}	5×10^{-5}
E. coli RII	5×10^{-3}	0.5

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

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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