

Change of host range in a resistance factor

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SUMMARY

An R factor for ampicillin and streptomycin resistance (AS) was identified in *Salmonella enteritidis*. The AS factor transferred freely to *Escherichia coli* K12, but only two of 260 K12(AS) clones from this cross would transfer AS to *S. typhimurium*, although all lines tested transferred it to *S. enteritidis* and K12. A study of one of the two exceptional lines revealed that it also transferred AS to *S. paratyphi* B, *S. thompson* and *S. anatum*. The R factor maintained its transferability when cycled between these serotypes and K12. Transfer to *S. enteritidis*, however, resulted in loss of the ability of AS to transfer to the heterologous serotypes, that is, it apparently became host specific for *S. enteritidis*. *S. paratyphi* B and *S. anatum* also imposed host specificity on AS, but *S. typhimurium* and *S. thompson* did not. The R factor would always enter *S. enteritidis*, whatever its previous salmonella host but, once it had done so, it became specific for *S. enteritidis*. AS could always transfer to K12, which did not seem to modify its host range. These phenomena are most easily explained by analogy with host-controlled modification of phage. Their possible significance in relation to apparent host specificity of R factors is discussed.

1. INTRODUCTION

The frequency of transfer of a resistance factor (R factor) depends on a number of features: the particular transfer factor-resistance determinant complex concerned; the recipient strain; the donor strain; the state of repression of the system, as well as non-specific features such as the cell concentration, the supply of metabolites and so on (Anderson, 1968). Other conditions being equal, a given R factor transfers at a constant frequency in a particular donor-recipient combination. Although it is known that some R factors transfer to some recipients with difficulty or not at all, a high order of change of host specificity of individual R factors does not seem to have been observed. We shall describe here such a change in an R factor introduced into various salmonella serotypes.

2. MATERIALS AND METHODS

The media for culture, and the experimental methods, were as described previously (Anderson & Lewis, 1965*a, b*). Unless otherwise indicated, crosses were routinely allowed to proceed overnight, that is for about 18 h, before mating

mixtures were plated on selective media for the isolation of drug-resistant recombinants. In crosses in which a salmonella was the donor and *Escherichia coli* K12 (= K12) the recipient, the donor was eliminated by incorporating 40 µg/ml of nalidixic acid in the antibiotic selective medium, the K12 recipient being a nalidixic acid-resistant mutant. When K12 was the donor and a salmonella the recipient, the donor was eliminated with colicin E2 (Anderson & Lewis, 1965*a*) to which salmonellae are resistant. Because crosses were at least 18 h in duration, frequencies of transfer were calculated in terms of the proportion of the recipient population that had received the R factor under study.

The bacterial strains used in these investigations are shown in Table 1.

Table 1. *Strains used in investigating transferability of the AS* resistance factor*

ERL No.	Description	Salmonella somatic group	Designation
12R927	Wild <i>S. enteritidis</i> phage type 8 carrying AS	D	<i>S. enteritidis</i> (AS)
14R525	<i>E. coli</i> K12F-Nx ⁺	—	K12
RT576	<i>S. typhimurium</i> phage type 36	B	<i>S. typhimurium</i>
27R5	<i>S. enteritidis</i> phage type 1	D	<i>S. enteritidis</i> 1
12R899	<i>S. enteritidis</i> phage type 8	D	<i>S. enteritidis</i> 8
27R98	<i>S. paratyphi</i> B phage type 1	B	<i>S. paratyphi</i> B
27R99	<i>S. thompson</i> phage type 1	C	<i>S. thompson</i>
27R100	<i>S. anatum</i>	E	<i>S. anatum</i>

* AS = Resistance to ampicillin and streptomycin.

Nx⁺ = Nalidixic acid resistant. Nalidixic acid was used for counter-selection in salmonella × K12 crosses.

3. RESULTS

The R factor to be discussed was identified in a strain of *Salmonella enteritidis* phage type 8 isolated from a boy of 8 years. Human infection with type 8 of *S. enteritidis* is common in England at present, and is known to originate mainly in broiler fowls. The great majority of strains are drug-sensitive, but the present strain, numbered 12R927, was resistant to ampicillin (A) and streptomycin (S). These resistances proved to be transferable *en bloc* to K12 at a frequency of about 3×10^{-3} . The R factor concerned will be designated AS. An attempt was then made to transfer the AS resistance factor from K12 to *S. typhimurium* phage type 36, which is used as a standard host for the examination of R factors (Anderson, 1966, 1968). Of 260 lines of K12(AS) used in efforts to transfer AS to *S. typhimurium*, only two yielded recombinants, although all lines tested could transfer AS to K12 (about 10^{-3}) and to *S. enteritidis* (about 10^{-4}). The frequency of AS transfer to *S. typhimurium* from one of the exceptional K12(AS) lines, numbered 27R7, was about 2×10^{-6} after 18 h, but rose to 4×10^{-1} when the cross was allowed to continue for a further 30 h. The line 27R7 also transferred AS to *S. paratyphi* B at a frequency of 10^{-2} , and to *S. thompson* and *S. anatum*, at frequencies of 3×10^{-1} and 4×10^{-1}

respectively, in 18 h. It transmitted AS to *S. enteritidis* 1 at a frequency that differed little from that of the K12 lines which did not transfer AS to the other salmonella serotypes.

Once transferred from 27 R 7 to *S. typhimurium*, *S. paratyphi* B, *S. thompson* and *S. anatum*, the AS resistance factor could transfer easily to K12 and thence again to the respective salmonella hosts at the following approximate frequencies: *S. typhimurium*, 10^{-4} ; *S. paratyphi* B, 3×10^{-2} ; *S. thompson*, 2×10^{-4} ; *S. anatum*, 10^{-3} . It would also transfer from the K12(AS) progeny derived from these salmonellae to *S. enteritidis* 1 and *S. enteritidis* 8, at frequencies between 10^{-4} and 3×10^{-4} . Subsequent transfer of AS to K12 and thence to *S. enteritidis* 1 occurred without difficulty. The K12(AS) progeny isolated from all *S. enteritidis* (AS) \times K12 matings, however, when crossed for 48 h with *S. typhimurium*, *S. paratyphi* B and *S. thompson*, did not transfer AS within the limit of sensitivity of the test (*c.* 10^{-8}), while the frequency of AS transfer to *S. anatum* was about 10^{-6} in a 48 h cross only when AS had already passed through that serotype. Passage into *S. enteritidis* had therefore greatly reduced the transferability of the AS resistance factor to the other salmonellae. These findings are summarized in Fig. 1.

As Fig. 1 shows, the initial AS transfer frequency from 27R7 to *S. typhimurium*, *S. paratyphi* B, *S. thompson* and *S. anatum* was high, although that to *S. typhimurium* required 48 h to reach its peak. Transfer of AS from each of these serotypes to K12 was also relatively frequent, as was further transfer of AS from K12 into the serotypes concerned. Once the AS factor had been introduced into *S. enteritidis*, however, its communicability pattern changed. It would pass freely from *S. enteritidis* into K12 and back into *S. enteritidis*, but had apparently lost the ability to enter the other salmonellae. An exception was *S. anatum*: the AS factor which had already passed through this serotype retained the ability to transfer to it from K12 after passage through *S. enteritidis*, though the frequency of transfer into *S. anatum* was greatly reduced.

It was thus evident that transfer of AS into *S. typhimurium*, *S. paratyphi* B, *S. thompson* and *S. anatum* had not impaired the ability of the R factor to enter *S. enteritidis*, the serotype in which it was originally identified. By contrast, transfer into *S. enteritidis* had seriously reduced the transmissibility of AS to the other salmonellae. Further experiments were therefore carried out to determine whether the transmissibility of AS to the other salmonellae used in these investigations was affected by introduction into each in turn. The results of these experiments are shown in Table 2.

The donors in columns 1, 2, 3 and 4 of Table 2 were K12(AS) lines resulting from the first passage of AS through the respective salmonella and thence into K12 (see Fig. 1). In column 5, in addition to one donor being the product of strain 12R927 \times K12, K12(AS) lines were included which resulted from transfer of AS from the K12(AS) strains of columns 1, 2, 3 and 4 into *S. enteritidis* and thence again into K12.

It is apparent from Table 2 that passage of AS through *S. paratyphi* B and *S. anatum*, as well as through *S. enteritidis*, resulted in the R factor becoming host

specific. While the host specificity conferred by *S. enteritidis* extended to all the other salmonellae used in this work, however, the ability of AS to transfer to *S. enteritidis* was always retained, irrespective of its last host. Two of the salmonellae, *S. typhimurium* and *S. thompson*, did not appear to confer narrow host specificity on AS. With the possible exception of 27 R7 and perhaps its sister line of K12, which has not yet been investigated, K12 seemed to carry AS without affecting the specificity it had acquired in its preceding salmonella host.

Table 2. *Transfer from K12(AS) into salmonellae*

Recipient strains	Donor strains				
	1 K 12(AS) (Ex. <i>S. typhi</i> - <i>murium</i>)	2 K 12(AS) (Ex. <i>S. para</i> - <i>typhi</i> B)	3 K 12(AS) (Ex. <i>S. thompson</i>)	4 K 12(AS) (Ex. <i>S. anatum</i>)	5 K 12(AS) (Ex. <i>S. enteri</i> - <i>tidis</i>) (directly or via 1, 2, 3 or 4)
<i>S. typhimurium</i>	+	-	+	-	-
<i>S. paratyphi</i> B	+	+	+	-	-
<i>S. thompson</i>	+	-	+	-	-
<i>S. anatum</i>	+	-	+	+	-*
<i>S. enteritidis</i>	+	+	+	+	+

+ = Transfer frequency 10^{-2} to 10^{-4} in overnight crosses.

- = No transfer detected in 48 h crosses.

* = Transfer frequency about 10^{-6} in 48 h crosses only when AS had previously passed through *S. anatum*.

4. DISCUSSION

The AS resistance factor is f_i^- and does not produce phage restriction in any of the salmonella hosts used in these experiments. It belongs to the group we now designate Class 1 of R factors, that is, the resistance determinants and the transfer factor which mobilizes them are covalently bonded, are transferred as an intact linkage group, and retain their transferability in new hosts. The prototypes of Class 1 are the R factors first discovered in Japan (review: Watanabe, 1963). Class 2 of R factors was discovered in *S. typhimurium* (Anderson & Lewis, 1965*a, b*): in this class the determinant and the transfer factor are independent of each other, occupy different cellular attachment sites, and transfer of one without the other is common.

We have so far been unable to demonstrate the presence of more than one R factor or transfer factor in the wild *S. enteritidis* strain 12R927 in which AS was identified, or in any subsequent host. Nor has dissociation of the AS determinant from its transfer factor been observed. It has therefore been assumed that only one Class 1 f_i^- R factor is concerned in the phenomena described.

Direct transfer of AS from *S. enteritidis* 12R927 to *S. typhimurium* could not be detected. The inability to enter *S. typhimurium* was thus a property of the over-

whelming majority of AS copies in the original host cells. Those able to enter other salmonellae could be regarded as having undergone host-range mutation or host-controlled (phenotypic) modification, to use phage terminology. If the process was due to host-range mutation, it must be assumed that 27 R 7, the K12(AS) line from which initial transfer to other salmonellae occurred, carried an assortment of mutants specific for particular salmonella hosts and that, when mutant selection by each of the respective hosts had occurred, for example, by *S. paratyphi* B, *S. anatum* and *S. enteritidis*, the progeny carried only that mutant, so that only AS copies specific for the respective serotype, and for *S. enteritidis*, were produced. Other mutants, for example those selected by *S. thompson* and *S. typhimurium*, would have a wide host range, and would therefore preserve their ability to enter other serotypes. In each case, however, crosses with *S. enteritidis* would result in selection of a mutant virtually specific for *S. enteritidis* only.

The phenomena could perhaps be more plausibly explained in terms of a process analogous to host-controlled modification (HCM) of phage (Luria & Human, 1952; Anderson & Felix, 1952; Bertani & Weigle, 1953; Weigle & Bertani, 1953). In HCM the host specificity of the phage is determined by the host strain in which it was last propagated. So long as this host remains unchanged, the specificity of the phage is preserved. Let us assume that its efficiency of plating (EOP) on a new host strain is 10^{-4} . Plaques picked from such a titration and propagated on the new strain will yield a phage specific for that strain, and with an EOP of perhaps 10^{-4} on the preceding host strain.

HCM takes place in a single cycle of phage growth in the host concerned. It can be repeated with a given phage in many different hosts. It is independent of host-range mutation, and the actual change appears to be determined, not by heterogeneity in the phage, but by exceptional host cells in which any phage particle can multiply.

In some HCM systems, certain bacterial strains will always give an EOP of 1 with the phage, irrespective of its specificity. A good example of this is found in the Vi-typing scheme for *S. typhi* (Craigie & Yen, 1938*a, b*), in which some typing phages are pure phenotypic modifications of Vi-phage II (Anderson & Fraser, 1955, 1956). In addition to their capacity to lyse their specific types with full efficiency, all these modified preparations retain an EOP of 1 on Vi-type A, which is the wild-type of *S. typhi*. Propagation of plaques from such a titration on Vi-type A yields phage A; this is the wild-type of Vi-phage II, and has an EOP of 1 on type A only.

The AS resistance factor was first identified in *S. enteritidis*. After transfer to K12 it could re-enter *S. enteritidis* with ease, but only exceptional lines of K12(AS) could transfer the R factor to other salmonellae. However, once transferred to these salmonellae, AS could be cycled between each serotype and K12 at relatively high frequency. In some instances, for example, with *S. paratyphi* B and *S. anatum*, the R factor had acquired specificity for those salmonellae; in others the ability to transfer to heterologous serotypes had been retained. However, all salmonellae carrying AS could transfer it to *S. enteritidis*. When this transfer occurred, the

original restricted host range of the plasmid was restored, and in general it could not be transferred to the other salmonella hosts.

If these phenomena are viewed as corresponding to HCM of phage, *S. enteritidis* could be regarded as occupying a position in this system analogous to that of Vi-type A of *S. typhi* in the Vi-typing scheme for that organism, in that *S. enteritidis* will accept AS from any serotype into which it has been transferred, irrespective of newly acquired specificity, but the host range of the plasmid is then restricted to *S. enteritidis*.

AS can always transfer to K12, irrespective of its preceding host, but K12 generally seems to act as an indifferent host, in which modification of AS does not occur, so that the plasmid retains the host range it possessed before entering K12. In this respect K12 might be regarded as corresponding in the AS system to r⁻m⁻ mutants described by Arber (1965) in relation to HCM of phage λ .

It could be argued that the AS in 27R7 was a mutant factor which possessed the capacity for adaptation to other salmonellae. A difficulty in accepting this hypothesis lies in the very high degree of host specificity of AS once it had entered *S. enteritidis*, which would imply that the mutant had lost its capacity for HCM. If the phenomenon is regarded as a form of HCM throughout this difficulty does not arise. It is advisable, however, not to pursue the analogy with HCM of phage too far, because, although *S. enteritidis* has been postulated to occupy a position comparable with that of Vi-type A in the Vi-phage typing scheme of *S. typhi*, the frequency of initial transfer of AS from 27R7 to *S. enteritidis* was about 10⁻⁴, which is lower than that to *S. paratyphi* B, *S. thompson* and *S. anatum* from the same donor. If these transfer frequencies are regarded as being analogous to phage EOP, *S. enteritidis* would not occupy a position in this respect similar to Vi-type A of *S. typhi*, on which all adaptations of Vi-phage II have an EOP of 1.

It is hoped to obtain more information on this and other aspects of the AS resistance transfer system with a derepressed mutant of AS, which will eliminate the delay in transfer imposed by repression.

We have not so far observed this phenomenon in other resistance transfer systems, so that it is impossible to speculate on its frequency. We have, however, found a number of R factors which, although they will transfer from the original salmonella or *E. coli* host into K12, will apparently not transfer into other standard hosts such as *S. typhimurium* type 36. An example of this is a tetracycline R factor from *S. panama*, which we have been unable to transfer from K12 into *S. typhimurium*. Similarly, the T- Δ resistance factor of type 29 of *S. typhimurium* (Anderson & Lewis, 1965*b*) has not so far been transferred into *S. panama* (E. S. Anderson, Grindley & White, unpublished observations). There is probably a considerable degree of intrinsic host specificity in R factors, and in the long term we would expect to find, in each of the various wild enterobacterial species, those R factors best qualified to infect, and survive in, the species concerned (Anderson, 1968). The findings described in this paper suggest, however, that there is also a mechanism for the modification of specificity of some R factors which may extend or limit the range of hosts they can invade, so that an R factor which appears to

have a particular host specificity may be basically identical with another which is apparently specific for a different host. This has important implications for the study of the ecology of R factors.

These observations are being extended and our further findings will be published in due course.

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