RESTRICTION AND MODIFICATION OF BACTERIOPHAGES BY R^+ STRAINS OF ESCHERICHIA COLI K12

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It has been previously reported by Watanabe and Okada (1964), Watanabe *et al.* (1964, 1966) that the *fi*$^-$ class of resistance transfer factors (R factors) restrict the growth of certain bacteriophages. Siccardi (1966) showed that restriction of phages BF23 and W31 was associated with *col I* resistance conferred by R factors, and that this was not confined to the *fi*$^-$ R factors alone. We have surveyed a total of 151 R factors (71 *fi*$^+$, 65 *fi*$^-$ and 15 for which the *fi* character was not determined) in the *Escherichia coli* K12 strain J5-3 F$^-$ *pro met* for restriction of the phages: λ, Ø80, P2, P1, BF23, W31, T3 and Ø1. The J5-3R$^+$ strains were kindly provided by Dr. Naomi Datta. The initial survey was carried out by spotting serial hundred fold dilutions of the J5-3 grown phage onto lawns of the R$^+$ cultures. The efficiencies of plating (e.o.p.) were then measured accurately by plating out suitable dilutions of phage. Table 1 presents the results of this survey. Of the 151 R factors tested, 59 (33 *fi*$^-$, 20 *fi*$^+$ and 6 others) restricted one or more of the phages. These 59 R factors can be divided into ten groups on the basis of the e.o.p. values shown in Table 1. It is interesting to note that some of these groups contain both *fi*$^-$ and *fi*$^+$ R factors.

Groups VI and IX show an e.o.p. of less than 1×10^{-3} for phage BF23 and a reduction in e.o.p. for phage W31. Six of the R factors in these two groups had been previously tested for colicin production and colicin resistance (Meynell, personal communication). All six were colicinogenic

TABLE 1.

Efficiency of plating of bacteriophages on R^+ strains of E. coli J5-3

R factor group	T3	W31	ø1	BF23	λvir	ø80	P2	P1	*No. of R factors fi⁻	fi⁺	not tested
I	1.0	1.0	1.0	1.0	$4x10^{-4}$	$1x10^{-4}$	$3x10^{-5}$	$1x10^{-1}$	-	1	-
II	1.0	1.0	1.0	1.0	$2x10^{-2}$	$7x10^{-3}$	$6x10^{-1}$	$7x10^{-4}$	3	6	-
III	1.0	1.0	1.0	1.0	$< 10^{-2}$	$3x10^{-2}$	1.0	1.0	1	3	-
IV	1.0	1.0	1.0	1.0	$< 10^{-2}$	1.0	1.0	1.0	-	-	1
V	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$3x10^{-1}$	$< 10^{-2}$	1.0	1.0	1.0	3	-	-
VI	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-2}$	1.0	1.0	1.0	1	-	-
VII	$8x10^{-1}$	$7x10^{-3}$	$7x10^{-1}$	$5x10^{-1}$	1.0	1.0	1.0	$7x10^{-7}$	-	2	-
VIII	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$	$1x10^{-1}$	1.0	1.0	1.0	1.0	2	1	1
IX	$7x10^{-1}$	$5x10^{-1}$	$8x10^{-1}$	$< 10^{-3}$	1.0	1.0	1.0	1.0	6	3	2
X	$1x10^{-1}$	$1x10^{-1}$	$1x10^{-1}$	1.0	1.0	1.0	1.0	1.0	17	4	2
others	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	32	51	9

The e.o.p. values are based on averages of at least two experiments with each member of a group. The e.o.p. is indicated as less than ($<$) a certain value in those cases where, because of abnormal plaque morphology, plaque counts were subject to considerable error.

*The fi character of the R factors was determined by Dr. Naomi Datta and Dr. Elinor Meynell.

for col Ib and col I resistant. Restriction of these phages associated with col I resistance has also been found by Siccardi (1966).

A number of e.o.p. values for phages T3, W31, Ø1 and BF23 reported in Table 1, though less than 1.0, are exceptionally high (1×10^{-1} to 8×10^{-1}). More commonly the e.o.p. of host modified phage on restricting host strains is reduced by several log. units (Lederberg 1957). However, Arber (1966) has shown that phage fd.B plates on B (P1) with an e.o.p. of 3×10^{-1}, and is host modified by it. In the examples cited in Table 1 there is some reduction in plaque size and, with the so-called female specific phages (T3, W31 and Ø1), there is a variation in plaque morphology (e.g. crenelation of the halo, or no halo). This effect would not be expected if, after the first round of infection, the phage was host modified and subsequent rounds of infection were normal. It is more likely that the reduction in e.o.p. and plaque size are due to one or more of several factors, for example reduction in burst size, inefficient adsorption, and slightly impaired transmission. Watanabe et al. (1966) have already reported a reduced burst size for T1 in an R^+ host.

In many instances where the e.o.p. recorded in the Table 1 is less than 1×10^{-2} and the phage is not host modified it has been possible to isolate mutants, which are no longer restricted, from plaques with normal morphology.

We have demonstrated host modification of phages λ, Ø80 and P2 in group I, and of λ, Ø80 and P1 in group II. Host modification of these phages is group specific in that phage grown on group I is still restricted by group II (and vice versa) and also in that phage grown on any member of group II now plates efficiently on any other member of the group, (Table 2). In addition, the phages λ, Ø80 and P1 grown on either group I or II, still plate inefficiently on the other restricting groups (see Table 1). Group II may be identical with the R factors found by Watanabe et al.(1964, 1966) which host modify λ, and an R factor which restricts but does not modify λ reported by them may belong to one of our groups V or VI.

Table 2.

Host modification of phages λ, ø80, P1 and P2 in E. coli J5-3R+ strains

Phage	R factor		
	none	group I (1 R factor)	group II (9 R factors)
λ.J5-3	1.0	$4x10^{-4}$	$2x10^{-2}$
λ. group 1	1.0	1.0	$1x10^{-2}$
λ. group II	1.0	$2x10^{-4}$	1.0
ø80.J5-3	1.0	$1x10^{-4}$	$7x10^{-3}$
ø80. group 1	1.0	1.0	$4x10^{-3}$
ø80. group II	1.0	$1x10^{-4}$	1.0
P2.J5-3	1.0	$3x10^{-5}$	-
P2. group I	1.0	1.0	-
P1. J5-3	1.0	-	$7x10^{-4}$
P1. group II	1.0	-	1.0

The figures in the table represent approximate e.o.p. values. The e.o.p. for phages grown on group II are expressed as an average for all members of the group.

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References

Arber, W. (1966) J. Mol. Biol. 20, 483.
Lederberg, S. (1957) Virology 3, 496.
Siccardi, A. G. (1966) Genet. Res. Camb. 8, 219.
Watanabe, T. and M. Okada (1964) J. Bacteriol. 87, 727.
Watanabe, T., H. Nishida, C. Ogata, T. Arai and S. Sato (1964) J. Bacteriol. 88, 716.
Watanabe, T., T. Takano, T. Arai, H. Nishida and S. Sato (1966) J. Bacteriol. 92, 477.