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Transduction of Various R Factors by Phage P1 in *Escherichia coli* and by Phage P22 in *Salmonella typhimurium*

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R factors fi^+ and fi^-, with various combinations of drug-resistance markers and isolated from independent sources, were transduced by phage P1kc in *Escherichia coli* and by phage P22 in *Salmonella typhimurium*. Usually the entire R factor was transduced by P1kc in *E. coli*, as indicated by the absence of segregation of the drug-resistance markers from their conjugal transferability. In contrast, the patterns of segregation of the drug-resistance markers and their conjugal transferability differed considerably among various R factors after transduction by P22 in *S. typhimurium*. Transduction frequencies varied among R factors in both transduction systems.

R factors, or episomal drug-resistance factors, can be transferred among various enteric bacteria both by conjugation and by transduction. We reported previously on the transduction of two R factors [one (222) with the markers of resistance to sulfanilamide (SU), streptomycin (SM), chloramphenicol (CM), and tetracycline (TC), and the other (R_6) with the markers of resistance to SU, SM, CM, TC, and kanamycin-neomycin] in *Escherichia coli* K-12 by phage P1kc and in *Salmonella typhimurium* LT-2 by phage P22 (17, 21). Both of these R factors usually were transduced entirely by P1kc in *E. coli*, and most of the transductants obtained in *E. coli* retained conjugal transferability of their drug-resistance markers. In contrast, the drug-resistance markers of the R factors invariably segregated into the TC marker and the other drug-resistance markers after transduction in *S. typhimurium* by P22. Furthermore, a majority of the drug-resistant transductants obtained in *S. typhimurium* were unable to transfer their drug-resistance markers by conjugation. These "defective" R factors obtained by transduction with phage P22 were assumed to be integrated into the *Salmonella* chromosome (17), as was later shown to be the case by Dubnau and Stocker (4), at least in some drug-resistant transductants. On the basis of the segregation patterns of the drug-resistance markers and the determinants of autonomous replicability and conjugal transferability of the transduced R factors, we have presented tentative genetic maps of R factors (13). The genetic region of the R factors that controls episomal functions, such as autonomous replication and conjugal transferability, was designated the resistance transfer factor (RTF; 13). The linkage patterns of the drug-resistance markers and RTF were quite similar in R factors 222 and R_6, except that R_6 has a kanamycin-neomycin resistance marker, whereas 222 does not (21). Both of these R factors were of the fi^+ type, which inhibits the formation of F pili (3) by the sex factor F (10, 11, 15, 18, 20). We report here further studies on the transduction of R factors from independent sources, in which various combinations of drug-resistance markers were employed to determine linkage patterns. As a result of this study, it is apparent that the linkage relationships of the drug-resistance markers and RTF are not necessarily uniform among various R factors, even though they have the same combinations of drug-resistance markers.

MATERIALS AND METHODS

Media and antibiotics. These were the same as those described in a previous paper (16).

Strains of bacteria and phages. These were identical to those employed previously (17, 21). Phage P1kc was used for transduction in *E. coli* CSH-2 (methionine-requiring, F^- substrain of K-12), and phage P22 was used for transduction in *S. typhimurium* LT-2.

Strains of R factors. All of the R factors, except K-R_3 and K-TC, were derived originally from *Shigella* strains isolated from independent epidemics (Table 1). R factors K-R_3 and K-TC are spontaneous segre-

gants (deletion mutants) isolated from R factor K in *S. typhimurium* LT-2 (13, 19).

Transfer of R factors by conjugation. The methods of transfer of R factors by mixed cultivation and by selection of recipient cells that acquired the R factors have been described in previous papers (14, 16).

Transduction of R factors. The methods of transduction of R factors by phage P1kc in *E. coli* and by phage P22 in *S. typhimurium* have been described in previous papers (14, 17). Phage P1kc was propagated on *E. coli* CSH-2 strains carrying R factors by the confluent lysis plate method described by Lennox (9). Phage P1kc was allowed to absorb to washed cells of the recipient (CSH-2) at 37 C for 20 min in saline adjusted to *p*H 7.0 and containing 0.0025 M $CaCl_2$. The phage-infected bacteria were washed once by centrifugation in the cold, and centrifuged bacteria were suspended in Penassay Broth (Difco) and incubated at 37 C with aeration. Phage P22 was grown on *S. typhimurium* LT-2 strains carrying R factors in Penassay Broth. A transducing lysate of P22 was added to a Penassay Broth culture of the recipient (LT-2) and aerated at 37 C. The phage-infected recipient cultures in both transduction systems were aerated at 37 C for 30 min before plating on selective media. The P1kc-infected *E. coli* cells were then plated on $CaCl_2$-free Lennox agar (9) containing each antibiotic, and the P22-infected *S. typhimurium* cells on nutrient agar containing each antibiotic. The concentrations of the drugs incorporated into selective media were 25 μg/ml for CM and TC, 10 μg/ml for SM, and 500 μg/ml for SU. The drug-resistant transductant colonies were scored after incubation of the plates for 24 hr at 37 C. In each transduction experiment, the sterility of the transducing phage lysate was tested, and the titer of the unadsorbed phage was determined from supernatant fluids centrifuged of phage-infected recipients. The drug-resistance markers and conjugal transferability of drug-resistant transductants were studied with the methods described previously (14, 17).

TABLE 1. *R factors used in this study*[a]

R factor strain	Drug-resistance markers and levels of drug resistance	*fi*[b]
N-1	SU (>500), Sm (>25, <50), Tc (>100, <200)	−
N-3	SU (>500), Sm (<25, >50), Tc (>25, <50)	−
N-6	SU (>500), Sm (>75, <100), Tc (>25, <50)	+
N-9	SU (>500), Sm (>75, <100), Tc (>25, <50)	+
R-15	SU (>500), Sm (>75, <100)	−
S-a	SU (>500), Sm (>10, <25), Cm (>100, <200)	−
S-b	SU (>500), Sm (>10, <25), Cm (>100, <200), Tc (>100, <200)	+
K	SU (>500), Sm (>10, <25), Cm (>100, <200), Tc (>100, <200)	+
K-R_3[c]	SU (>500), Sm (>10, <25), Cm (>100, <200)	+
K-TC[c]	TC (>100, <200)	+

[a] SU, SM, CM, and TC are the abbreviations of sulfanilamide, streptomycin, chloramphenicol, and tetracycline, respectively. Levels of drug resistances conferred by R factors were determined on nutrient agar with *Escherichia coli* CSH-2 as a host.

[b] Presence (+) and absence (−) of suppression of the function of F of *E. coli* K-12.

[c] Spontaneous segregants from R factor K.

RESULTS

Transduction of R factors by phage P1kc in E. coli CSH-2. The frequencies of transduction by phage P1kc in *E. coli* CSH-2 differed considerably among various R factors (Table 2). No correlation was observed between the frequencies of transduction and the *fi* type of R factors. Segregation of the drug-resistance markers usually did not occur in any of the R factors regardless of their drug-resistance markers and *fi* types. Furthermore, most of the drug-resistant transductants obtained from any of the R factors were able to transfer their drug-resistance markers by conjugation. In the transduction of R factor K, the yield of the TC, SU, SM, and CM classes was lower in the selection with TC than with CM. This discrepancy shows that the selection with TC failed, for some unknown reason, to detect the transductants which could be recovered with the selective CM system.

Transduction of R factors by phage P22 in S. typhimurium LT-2. As in the case of P1kc, the frequencies of transduction with phage P22 in *S. typhimurium* LT-2 differed considerably among various R factors (Table 3). Again, the transduction frequencies did not show any correlation with the *fi* type of R factors. It is interesting to note here that the TC marker was cotransduced at high frequencies with the SU and SM markers in R factors N-3 and N-6, in contrast to our previous results with R factors 222 and R_6 in which the TC marker was invariably segregated from the other drug-resistance markers (17, 21). Furthermore, all of the drug-resistant transductants obtained with R factor S-a could transfer their drug-resistance markers by conjugation. Similar results occurred with a high proportion of the drug-resistant transductants obtained with R factors N-3, N-6, and N-9. None of the drug-resistant transductants obtained with K-R_3 could transfer their drug-resistance markers by conjugation, unlike those obtained from the S-a factor, which carries the same drug-resistance markers as K-R_3. A majority of the TC-resistant transductants obtained with N-9 were able to transfer their drug-resistance markers by conjugation,

TABLE 2. *Frequencies and types of drug-resistant transductants obtained in transduction of R factors by phage P1kc in Escherichia coli K-12*[a]

R factor	Selected marker	Drug-resistance markers of transductants	Frequency of transductants	t^+ per markers studied[c]
N-1	TC	TC, SU, SM	6.7×10^{-9}	50/50
N-3	TC	TC, SU, SM	7.1×10^{-7}	50/50
N-6	TC	TC, SU, SM	1.0×10^{-10}	50/50
N-6	SM	SM, SU, TC	1.0×10^{-10}	49/49
N-9	TC	TC, SU, SM	4.4×10^{-10}	49/49
N-9	SM	SM, SU, TC	3.2×10^{-10}	9/11
R-15	SM	SM, SU	3.2×10^{-6}	50/50
S-a	CM	CM, SU, SM	2.5×10^{-6}	30/30
S-a	SM	SM, SU, CM	2.8×10^{-6}	30/30
S-b	TC	TC, SU, SM, CM	4.5×10^{-6}	44/44
S-b	TC	TC	9.0×10^{-7}	6/6
K	CM	CM, SU, SM, TC	1.6×10^{-5}	59/59
		CM, SU, SM	2.9×10^{-7}	1/1
K	TC	TC, SU, SM, CM	4.5×10^{-6}	55/55
		TC	4.1×10^{-7}	5/5
K-R_3	CM	CM, SU, SM	5.6×10^{-5}	38/38
K-TC	TC	TC	1.4×10^{-6}	50/50

[a] SU, SM, CM, and TC are the abbreviations of sulfanilamide, streptomycin, chloramphenicol, and tetracycline, respectively. Selected markers other than those listed in this table were tested, but selection with them was not clear-cut. The method of transduction is described in the text. The multiplicity of input of phage in transduction was about 0.1. The adsorption rate of phage in these conditions ranged between 94 and 96%.

[b] Frequencies of transductants are expressed in values per adsorbed active phage.

[c] The possession of conjugal transferability of the drug-resistance markers is indicated by t^+.

TABLE 3. *Frequencies and types of drug-resistant transductants obtained in transduction of R factors by phage P22 in Salmonella typhimurium LT-2*[a]

R factor	Selected marker	Drug-resistance markers of transductants	Frequency of transductants[b]	t^+ per markers studied[c]
N-1	TC	TC	2.7×10^{-9}	1/109
	SU	SU, SM	9.5×10^{-7}	0/180
N-3	TC	TC, SU, SM	1.9×10^{-9}	72/159
		TC	2.0×10^{-9}	5/176
N-3	SU	SU, SM, TC	2.7×10^{-10}	16/21
		SU, SM	8.0×10^{-9}	5/6
N-6	TC	TC, SU, SM	1.7×10^{-9}	3/6
		TC	1.2×10^{-9}	0/4
N-9	TC	TC, SU, SM	1.0×10^{-10}	1/1
		TC	3.9×10^{-9}	42/45
S-a	CM	CM, SU, SM	1.6×10^{-5}	60/60
S-b	CM	CM, SU, SM	1.2×10^{-7}	0/60
S-b	TC	TC	8.1×10^{-9}	0/50
K	CM	CM, SU, SM	5.9×10^{-7}	0/200
K	TC	TC	2.6×10^{-8}	1/200
K-R_3	CM	CM, SU, SM	1.0×10^{-6}	0/180
K-TC	TC	TC	1.2×10^{-8}	1/120

[a] SU, SM, CM, and TC are the abbreviations of sulfanilamide, streptomycin, chloramphenicol, and tetracycline, respectively. Selected markers other than those listed in this table were tested, but selection with them was not clear-cut. The method of transduction is described in the text. The multiplicity of input of phage in transduction was about 10. The adsorption rate of phage in these conditions ranged between 78 and 85%.

[b] Frequencies of transductants are expressed in values per adsorbed active phage.

[c] The possession of conjugal transferability of the drug-resistance markers is indicated by t^+.

but almost none of the TC-resistant transductants obtained with N-1, K, and K-TC had conjugal transmitting ability.

DISCUSSION

Our structural models of R factors 222 and R_6, which were previously presented on the basis of transductional analysis of the drug-resistance markers and on the basis of the genetic determinants of autonomous replication and conjugal transfer, were compatible with the results of spontaneous segregation (or deletion) of the drug-resistance markers (19, 21). On the other hand, Anderson and Lewis (1, 2) claimed that R factors are actually composed of two separate units, "drug-resistance plasmids" and "transfer factors." Our previous results on the transduction of R factors 222 and R_6 appeared contradictory to the theory of Anderson, because the drug-resistance markers and their conjugal transferability can be cotransduced by phage P1kc. The results of further investigation by transduction of more R factors from different sources, which are reported here, confirm our previous results and are again in contradiction to the theory of Anderson. We have to conclude, therefore, that Anderson's report probably dealt with exceptional cases. His drug-resistance plasmids may be defective R factors comparable to those that were reported by Hirota, Fujii, and Nishimura (6), in view of the finding that the nontransmissible R factor mutants described by these authors can be complemented by the sex factor F for recovering conjugal ability. It is not unreasonable to assume that R factors carrying a defective RTF may be complemented by a nondefective RTF in a similar fashion.

The fact that most of the drug-resistant transductants obtained with any R factors by phage P1kc in *E. coli* are able to transmit their drug-resistance markers by conjugation is in striking contrast to the result that many of the drug-resistant transductants obtained by phage P22 lack conjugal transmitting ability of their drug-resistance markers. Transduction of drug-resistance markers without an RTF may occur also with P1kc, but such transduced, defective R factors may become abortive unless they are integrated into host chromosomes or form heterogenotes with the help of the transducing phage genome.

The origin(s) of R factors is not known, although we have presented the hypothesis that they might be comparable to F′ factors. It is conceivable that the drug-resistance markers of the R factors might have been picked up by an episome RTF from the chromosomes of some unknown bacteria (13). The transduction data, which are reported here as well as in previous papers, might give some suggestions to the solution of this difficult question. Our results indicate that the linkage relationships among various drug-resistance markers and RTF are not necessarily the same among various R factors. These results suggest that the R factors with few drug-resistance markers might have been produced as a result of deletion of markers from R factors with a greater number of drug-resistance markers. This interpretation is supported by our epidemological findings on the development of R factors (13). In Japan, multiple drug-resistance R factors appeared prior to R factors with fewer drug-resistance markers. It must be kept in mind, however, that R factors with multiple drug-resistance markers can be produced by genetic recombination of R factors carrying only a few drug-resistance markers (19).

The differences in the frequencies of transduction among various R factors probably result from differences in their molecular sizes. Ikeda et al. (7, 8) have shown that the size of the P1 transducing fragments equals the molecular size of the P1 chromosome. A similar situation is likely also with phage P22 (H. Ozeki, *personal communication*). It has already been established (5, 12) that the genetic material of R factors is deoxyribonucleic acid (DNA). If the size of the DNA of an R factor does not approximate the size of the DNA transducing particle, the frequency of transduction should be reduced.

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