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Interaction of *Pseudomonas* and *Enterobacteriaceae* Plasmids in *Aeromonas salmonicida*

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We observed that *Aeromonas salmonicida* ARO200 will maintain either or both the *Pseudomonas* R-factor, pMG1, and *Enterobacteriaceae* R-factors. This bacterial strain, therefore, provides a unique genetic background wherein the host ranges of *Pseudomonas* and *Enterobacteriaceae* plasmids overlap. Comaintenance of these plasmids resulted in behavior of plasmid aggregates that allowed transfer of R-determinants beyond the host range of the parent plasmid. We observed that the ARO200 genetic background facilitated the redistribution of R-determinants among unrelated and conjugally noninterfertile gram-negative bacteria. Aberrant behavior resulting in the deletion of R-determinants for plasmids singly maintained in ARO200 was also observed. Plasmids studied included RP1, R702, IncP; Rs-a, IncW; R192.7, IncFII; R64-11, IncI; R390, IncN; and R6K, IncX.

Aeromonas salmonicida, a member of the bacterial family *Pseudomonadaceae*, is a gram-negative, nonmotile, rod-shaped bacterium. It is facultatively anaerobic, asporogenous, facultatively psychrophilic, capable of fermenting carbohydrates with the production of acid, and oxidase positive (5). Accordingly, its metabolism demonstrates features of disparate groups of organisms including the terrestrial saprophytic pseudomonads and the *Enterobacteriaceae*. *A. salmonicida* has a wide geographical distribution, primarily in freshwater fish (26, 27), particularly *Salmonidae* (8, 9, 10), wherein it may produce epizootic furnuculosis and bacteremia. R-factors have been found in *A. salmonicida* strains isolated from nature that have been transferable to *Aeromonas hydrophila*, *Escherichia coli*, and other *A. salmonicida* (2, 24). This is not surprising in view of the use of antibiotics in intensive fish-farming (27).

We considered that the expression by *A. salmonicida* of metabolic activities characteristic of either pseudomonads or *Enterobacteriaceae* might also be accompanied by a corresponding ability to host R-factors whose host range, based on previous observations, is limited to either pseudomonads (4, 16, 25) or *Enterobacteriaceae* (7). Thus, co-maintenance by aeromonads of plasmids with limited host range would allow for exchange of drug resistance determinants between plasmids, followed by their redistribution to bacteria unable to host one of the parent plasmids. In this regard, aeromonads would provide a common genetic background for the interaction of R-factors that otherwise would not coexist in a given bacterium.

Unusual behavior of plasmid aggregates in some bacterial hosts has been reported, i.e., a dramatic change in the integrity of the plasmid aggregate and a change in the cellular content of the plasmid under some growth conditions (15). A plasmid aggregate has been defined by Clowes (6) as coexisting plasmids that are not covalently linked and under most circumstances transfer and cure independently. With these observations in mind, we noticed many examples of co-transfer of the aggregate constituent plasmid R-determinants during our preliminary studies with *A. salmonicida*. These observations were explicable only on the basis of the transfer of a plasmid (or some of its R-determinants) beyond its presumed normal host range. This in turn focused our attention on the possibly unique behavior of plasmid aggregates in *A. salmonicida* with respect to the transfer of R-factors beyond the host range of the parent plasmid when maintained in its specific host.

For this work, we used R-factors encoding antibiotic resistances previously noted for the bacterial hosts studied. Therefore, no novel antibiotic resistance phenotypes have been produced as a result of this study.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. The relevant properties of the bacteria, plasmids, and phages used are listed in Table 1.

Media. Complex medium (TN) and minimal medium (VBG) were prepared as described previously

TABLE 1. *Bacteria, plasmids, and phages used*

Strain, plasmid, or phage	Relevant characteristics[a]
Escherichia coli	
J53	Pro⁻ Met⁻
ROE531	Met⁻ (Pro⁺ J53 transductant)
Pseudomonas aeruginosa	
PA038	*leu-38*
PA0222	*met-28, trp-6, lys-12, his-4, pro-82, ilv-225*
PAT1227	*his-404, res-1102* (Res⁻ Mod⁺)
Aeromonas salmonicida	
ARO200	Met⁻ Arg⁻ ATCC 14174
Plasmid[b]	
RP1	Cb^r, Tc^r, Nm/Km^r, IncP, broad host range
R702	Sm^r, Su^r, Nm^r, Tc^r, Hg^r, IncP, broad host range
Rs-a	Sm^r, Su^r, Nm^r, Cm^r, IncW, broad host range
R390	Sm^r, Su^r, Cb^r, Cm^r, Tc^r, IncN, limited host range
R192.7	Sm^r, Cm^r, Tc^r, Inc FII, limited host range
R6K	Sm^r, Cb^r, IncX, limited host range
R64-11	Sm^r, Tc^r, IncI, limited host range
pMG1	Sm^r, Su^r, Gm^r, Hg^r, IncP2, limited host range
Phage	
PRR1	Pilus specific, P plasmid incompatibility group-specific phage (23)
PRD1	P, N, W plasmid incompatibility group-specific phage (22)
f2	Pilus specific, FII plasmid incompatibility group phage (18)
Ike	N plasmid incompatibility group-specific phage (17)

[a] Plasmid markers designate resistances to carbenicillin (Cb^r), chloramphenicol (Cm^r), gentamicin (Gm^r), kanamycin (Km^r), neomycin (Nm^r) mercuric chloride (Hg^r). Inc denotes the plasmid incompatibility group. The sources of these bacterial strains and plasmids have been given in previous publications (20, 22, 23).

[b] All plasmids used in this study were maintained in J53 except pMG1, which was maintained in PA038.

(20, 21). When complex medium with brain heart infusion (BHI) was used, it contained (per liter) 36 g of BHI (Difco). BHI medium was solidified by the addition of 20 g of agar. Antibiotic supplements are as described in the tables. Mercury resistance was determined using BHI supplemented with $HgCl_2$ to a final concentration of 30 μg/ml. When nutritional selection against auxotrophic donors was done, amino acid or purine requirements were satisfied by the addition of these components to a final concentration of 20 μg/ml.

Mating and testing of exconjugants. All matings involving ARO200 were done in BHI broth medium. For this, BHI broth medium was inoculated with overnight growth on TN agar (for *E. coli* and *Pseudomonas aeruginosa*) or BHI agar (for ARO200) containing the appropriate antibiotic for the strain containing a plasmid. Usually, these broth cultures were incubated for 3 h with agitation at 30°C for ARO200 and 37°C for *E. coli* or *Pseudomonas aeruginosa*. Inoculation was adjusted to result in approximately 10^8 cells per ml of BHI broth culture after 3 h of growth. Donor and recipient cells were mixed 1:1 and incubated at 4 h at 30°C. Mating mixtures were centrifuged at ambient temperature, and cell pellets were suspended to $^1/_{10}$ the original volume in 0.01 M phosphate buffer (pH 7.0). Appropriate dilutions of the mating mixture were made and plated onto VBG supplemented with the nutrients required by the recipient and one of the antibiotics whose resistance was determined by the plasmid. Plates were incubated for 48 h at 30°C (ARO200 exconjugants) or 37°C (*E. coli* or *P. aeruginosa* exconjugants) for quantitative estimates of mating frequencies. Matings not involving ARO200 were the same except TN broth medium was used, all incubations were at 37°C, and the mating time was 2 h. For purification and testing of exconjugants, colonies were picked into liquid suspension and streaked out for single-colony isolation on solid media containing the appropriate supplements. When doubles, i.e., bacteria containing two plasmids, were used for donors in mating experiments, they were grown on the appropriate complex media supplemented with the antibiotics necessary for the maintenance of both plasmids.

RESULTS

Recipient characteristics of *A. salmonicida*. We considered that the behavior in ARO200 of known broad host range plasmids might provide a convenient reference for evaluating the performance of plasmids whose host range is generally considered restricted to either the *Enterobacteriaceae* or *Pseudomonas* bacterial strains. The R-factors used and their mating behavior into ARO200 are shown in Table 2. Selection of exconjugants containing a plasmid (R⁺) was done using medium containing selective antibiotic corresponding to one of at least three of the resistances encoded by the R-factor. Three media containing different antibiotics were used in this experiment to detect possible alterations in R-factor properties occurring in ARO200.

Data in Table 2 show no significant variation in recipient ability by ARO200 when comparing either the R-factors used or the selective antibiotic for a particular R-factor. However, when R⁺ exconjugants from these matings were purified on selective medium used for their isolation and subsequently tested for maintenance of nonselected R-determinants, loss of nonselected markers was observed. Specifically, ARO200-(RP1) selected on medium containing carbenicillin were observed to have lost Nm^r and Tc^r. Similarly, ARO200(Rs-a) exconjugants selected

TABLE 2. *Transfer of broad host range R-factors to ARO200*

Donor	Selection[a]	Exconjugants per donor
J53(RP1)	Cb^r	5×10^{-4}
	Nm^r	1×10^{-3}
	Tc^r	1×10^{-3}
J53(R702)	Sm^r	3×10^{-4}
	Nm^r	5×10^{-4}
	Tc^r	5×10^{-4}
J53(Rsa)	Sm^r	2×10^{-4}
	Nm^r	9×10^{-6}
	Cm^r	3×10^{-4}

[a] Selection for the transfer of R-factors was on minimal medium containing either 500 μg of carbenicillin, 50 μg of streptomycin, 50 μg of neomycin, 5 μg of chloramphenicol, or 2 μg of tetracycline per ml.

for the acquisition of Nm^r were observed to have lost Cm^r but not Sm^r. Exconjugants isolated, purified, and tested from other selective media showed the nonselected maintenance of relevant R-determinants. We also tested for the maintenance of these R-factors in the absence of antibiotics. When colonies derived from antibiotic-free broth cultures were tested, many were cured of their R-determinants. Thus, these broad host range R-factors are apparently significantly less stable in ARO200 than we have observed previously in other gram-negative bacteria (21).

Data for the transfer of narrow host range R-factors representative of those isolated from antibiotic-resistant *Enterobacteriaceae* or *P. aeruginosa* are shown in Table 3. Again, as in the previous experiment, we selected for either of at least two antibiotic resistances to allow an estimate of the nonselected maintenance of these R-determinants. With these R-factors, unlike the broad host range R-factors in Table 2, differences in transfer were associated with the identity of the selective antibiotic. With the N incompatibility group plasmid, R390, no carbenicillin-resistant (Cb^r) exconjugants were obtained, although all exconjugants selected on medium containing streptomycin were found to be Cb^r. We consider that this may reflect a delay in Cb^r expression encoded by R390 in ARO200. An analagous result was observed for the expression of tetracycline resistance (Tc^r) by R64-11, an I incompatibility group R-factor. R64-11 was also observed to be unstable in the absence of streptomycin selection. The other R-factors listed in Table 3 were stable in the absence of selective antibiotic, although loss of an R-determinant was occasionally observed (approximately on the order of 1 colony per 16 tested).

The P-2 incompatibility group, *Pseudomonas* plasmid pMG1 (16), was stable in the complete absence of selective antibiotic and both streptomycin and gentamicin resistance were co-transferred independently of the selective antibiotic. However, all R^+ exconjugants from these matings lost mercury resistance previously encoded by pMG1 in pseudomonads. This was confirmed by testing PAO38(pMG1) exconjugants from retransfer experiments studied in Table 4 (data not shown).

R-factor donor ability of *A. salmonicida*. Plasmid-specific phages have been described for several of the incompatibility groups represented by R-factors used in this study. Therefore, the susceptibility of R^+ ARO200 isolates was determined to indicate the expression of plasmid-encoded receptors. Phage sensitivities appropriate to the R-factors used here are as follows: phage PRR1 for the P incompatibility group plasmids, RP1 and R702; phage PRD1 for P, W, and N incompatibility group plasmids RP1, R702, Rs-a, and R390, respectively (22, 23); phage Ike for the N incompatibility group plasmid, R390 (17); and phage f2 for the FII incompatibility group plasmid, R192.7 (18). When bacterial lawns on BHI medium inoculated with cells previously grown on selective medium were tested by spotting high-titer phage suspensions, no sensitivity was detected. Titer-increase tests, however, showed significant (>50-fold) growth of phage. Phages f2 and PRR1 are known to be pilus specific, and thus resistance to these phages might indicate either repression, deletion, or reduced expression of R-

TABLE 3. *Transfer to ARO200 of R-factors with narrow host range*[a]

Donor	Selection	Exconjugants per donor
J53(R390)	Cb^r	$<1 \times 10^{-8}$
	Sm^r	2×10^{-3}
	Cm^r	1×10^{-3}
J53(R192.7)	Sm^r	1×10^{-3}
	Tc^r	4×10^{-6}
	Cm^r	2×10^{-4}
J53(R6K)	Cb^r	1×10^{-3}
	Sm^r	1×10^{-3}
J53(R64-11)	Sm^r	1×10^{-3}
	Tc^r	$<1 \times 10^{-8}$
PA038(pMG1)	Gm^r	2×10^{-7}
	Sm^r	2×10^{-7}

[a] Selection of pMG1 exconjugants was on appropriately supplemented minimal medium containing either 25 μg of streptomycin or 5 μg of gentamicin per ml. Selection of other R^+ exconjugants was as described in Table 2.

TABLE 4. *Retransfer of R-factors from ARO200*[a]

ARO200(R+) donor	Recipient	Selection	Exconjugants per donor
RP1	J53	Cb^r	3×10^{-5}
		Nm^r	$<1 \times 10^{-8}$
		Tc^r	$<1 \times 10^{-8}$
R702	J53	Sm^r	9×10^{-7}
		Nm^r	2×10^{-7}
		Tc^r	$<1 \times 10^{-8}$
Rsa	J53	Sm^r	1×10^{-7}
		Nm^r	$<1 \times 10^{-8}$
		Cm^r	2×10^{-5}
R390	J53	Cb^r	2×10^{-5}
		Sm^r	1×10^{-6}
		Cm^r	5×10^{-5}
R192.7	J53	Sm^r	4×10^{-7}
		Tc^r	$<1 \times 10^{-8}$
		Cm^r	3×10^{-7}
R6K	J53	Cb^r	4×10^{-6}
		Sm^r	4×10^{-5}
R64-11	J53	Sm^r	3×10^{-7}
		Tc^r	$<1 \times 10^{-7}$
pMG1	PA038	Sm^r	7×10^{-5}
		Gm^r	5×10^{-7}

[a] Selection for R+ exconjugants was as described in the text and in Tables 2 and 3.

factor transfer functions in ARO200. To gain some estimate of the expression of transfer functions associated with the plasmids studied in ARO200, we next did the retransfer experiments shown in Table 4. All the frequencies of transfer indicated in Table 4 are low when compared with those for intraspecific mating between *Enterobacteriaceae* or among pseudomonads for pMG1. However, deletion of transfer functions for P, N, W, or FII group plasmids is precluded by these data. Therefore, diminished expression of transfer functions seems the most likely basis for phage insensitivity corresponding to low donor activity for R+ ARO200 strains. Our inability to recover R+ J53 exconjugants using some selective antibiotics probably cumulatively reflects diminished recovery peculiar to these R-determinants and also low donor activity on the part of ARO200 cells containing the R-factor in question.

For all R-factors in question, the R+ exconjugants showed a high incidence of R-determinant deletions that may have occurred during transfer from R+ ARO200 to either J53 or PAO38. R+ ARO200 donor cells were tested and showed the expected antibiotic resistances. Approximately 20% of the 16 or more R+ exconjugants tested for each mating had lost at least one nonselected R-determinant. However, the remaining R-determinants were stable in the complete absence of antibiotic selection. This result, taken with the tendency to delete R-determinants by ARO200 in newly formed R+ exconjugants, prompted us to consider that fragmentation of R-determinant linkage groups occurring in ARO200 would facilitate recombination between unrelated R-factors present simultaneously in this bacterium. To test this possibility we constructed ARO200 strains containing one *Enterobacteriaceae* R-factor and one pseudomonad R-factor. R-factor pMG1 was used as an example of the latter and accordingly paired with either R6K or R192.7.

These doubles designated ARO200(pMG1/R6K) and ARO200(pMG1/R192.7) were periodically transferred and maintained on medium containing a selective antibiotic for each of the plasmids present. After several serial propagations it was found that single selection for an R-determinant would allow for co-maintenance of the nonselected determinants. These strains were then used as donors for the matings shown in Table 5. The recipients, *E. coli* J53, *P. aeruginosa* PAO222, or *P. aeruginosa* PAT-1227, were chosen to detect transfer of an R-determinant outside of the host range of the parent R-factor. For example, R6K or R192.7 are not transferable to pseudomonads and thus transfer of either Cb^r encoded by R6K or Tc^r encoded by R192.7 to a pseudomonad may indicate transfer unique to the *Aeromonas* genetic background by mediation of the *Pseudomonas*

TABLE 5. *Transfer of hetero-R-determinants from ARO200 containing two plasmids of different host range*

Donor	Recipient	Selection[a]	Exconjugants per donor
ARO200(pMG1/R6K)	J53	Cb^r	1×10^{-5}
		Sm^r	3×10^{-7}
		Gm^r	1×10^{-7}
	PAO222	Cb^r	6×10^{-6}
		Sm^r	8×10^{-7}
		Gm^r	3×10^{-6}
ARO200(pMG1/R192.7)	J53	Gm^r	$<1 \times 10^{-8}$
		Sm^r	1×10^{-7}
		Cm^r	1×10^{-6}
	PAO222	Gm^r	4×10^{-6}
		Sm^r	1×10^{-6}
		Tc^r	7×10^{-5}
	PAT1227	Gm^r	1×10^{-6}
		Sm^r	2×10^{-6}
		Tc^r	2×10^{-4}

[a] Selection for the transfer of R-determinants into PAO222 or PAT1227 used minimal medium containing either 100 μg of tetracycline, 50 μg of streptomycin, or 25 μg of gentamicin per ml. Selection for Cb^r was as described in Table 2.

plasmid, pMG1. Neither R6K nor R192.7 is transferable from ARO200 to pseudomonads when singly present in *Aeromonas* (data not shown).

The data in Table 5 do indicate transfer of an "outside" R-determinant from *Aeromonas* doubles. For example, we observed transfer of gentamicin resistance (Gm^r) from ARO200(pMG1/R6K) to J53 and transfer of Cb^r derived from R6K to PAO222 along with Gm^r, the pMG1 R-determinant. The origin of streptomycin resistance (Sm^r) is indeterminant. However, in the latter instance, the maintenance of Cb^r by PAO222 R^+ exconjugants was lost in the absence of carbenicillin. Carbenicillin resistance derived from R6K was similarly and anomalously unstable when mated into J53 from ARO200(pMG1/R6K). This is unlike the stability observed for R6K when retransferred singly from ARO200(R6K) to J53.

The behavior of the ARO200(pMG1/R192.7) R-factor complex resembled the above in some respects. When R^+ J53 exconjugants were selected for Gm^r, no apparent transfer was detected, but when exconjugants selected for the acquisition of Sm^r were purified and tested, all were found to be Gm^r. However, none was Tc^r as specified by R192.7. In addition, the foregoing R^+ exconjugants selected for their Sm^r were also Cm^r, although this R-determinant was unstable in the absence of continued selection during serial propagation. On the other hand, Tc^r, presumably derived from R192.7, was stable in *Pseudomonas* PAO222 or PAT1227 exconjugants in the absence of selective antibiotic. It was also observed to be co-transferred from ARO200 to *Pseudomonas* whether exconjugants were selected initially for the acquisition of Sm^r or Gm^r. This behavior, then, is consistent with the transposition of Tc^r into the P-2 group plasmid pMG1 either in the *Aeromonas* donor population or in newly formed R^+ exconjugants which had received both pMG1 and R-determinants derived from R192.7 and mobilized by pMG1. We do not presently know the reason for the instability of Cb^r or Cm^r R-determinants after transfer promoted by pMG1 into J53 or PAO222.

Behavior of R-determinant aggregates derived from R^+ ARO200. We next attempted retransfer of aggregate R-determinants obtained from matings shown in Table 5. Typical strains produced during the matings shown in Table 5 were tested for their abilities to transfer R-determinants to *E. coli* ROE531 or *P. aeruginosa* PAO38. The results of these matings are shown in Table 6.

J53(pMG1/R6K) was Gm^r (derived from pMG1) and Sm^r, which may have originated in either pMG1 or R6K. As previously mentioned, these exconjugants were unstable for the maintenance of Cb^r derived from R6K. When one isolate was mated with PAO38 and R^+ exconjugants selected for gentamicin resistance, all were found to be streptomycin resistant. However, serial propagation on medium containing streptomycin resulted in the unexpected loss of gentamicin resistance. This result contrasts with the behavior of either ARO200(pMG1) or PAO38(pMG1), which show the nonselected maintenance of either Sm^r or Gm^r (unpublished observations).

TABLE 6. *Retransfer of R-determinants from R^+ ARO200 doubles*

Donor	Recipient	Selection[a]	Exconjugants per donor
J53(pMG1/R6K)	ROE531	Gm^r	$<1 \times 10^{-8}$
	PAO38	Gm^r	2×10^{-7}
PAT1227(pMG1/R192.7)	ROE531	Gm^r	6×10^{-7}
	PAO38	Gm^r	4×10^{-7}
PAO222(pMG1/R192.7)	ROE531	Gm^r	2×10^{-7}
	PAO38	Gm^r	4×10^{-7}

[a] Selection for the acquisition of Gm^r was as described in Table 3.

PAT1227(pMG1/R192.7) was Gm^r, Sm^r, and Tc^r. The Tc^r R-determinant presumably was derived from the enteric bacteria plasmid, R192.7. When this isolate was mated with ROE531, using gentamicin selection, purified R^+ ROE531 exconjugants were Gm^r and Sm^r. However, none was Tc^r. Furthermore, growth of these *E. coli* exconjugants on nonselective medium resulted in the loss of both Gm^r and Sm^r. Therefore, the R-factor transferred to ROE531 was highly unstable. When PAO38 was the recipient in these matings, Gm^r and Sm^r were co-transferred and stably maintained, but Tc^r was not. Thus, in this cross using *Psuedomonas* recipients as with ROE531 recipients, Tc^r was not mobilized from PAT1227.

Exconjugants from matings using PAO222-(pMG1/R192.7) as the donor behaved as described above for the R^+ PAT1227 donor strain. Therefore, in retrospect, the Tc^r determinant derived from R192.7 was mobilized from ARO200 to *Pseudomonas* strains but could not be subsequently retransferred either to *E. coli* or *Pseudomonas* recipients. A more curious anomaly occurring for exconjugants produced by matings shown in Table 6 is the unstable maintenance of R-determinants formerly originating in these hosts. For example, pMG1 is stable in *Pseudomonas* strains used here and also when singly present in ARO200. However, after co-maintenance of either R6K or R192.7 R-

determinants in ARO200 R^+ doubles, the R-factor aggregate complexes show unstable maintenance in subsequent bacterial hosts after their acquisition from R^+ ARO200. This instability seems to be further enhanced with subsequent partial retransfer of R-determinants to either *E. coli* or to *Pseudomonas* bacteria.

DISCUSSION

A salmonicida ARO200 is able to maintain disparate plasmids (incompatibility groups, P, P2, W, N, FII, X, and I) and thus provides a unique example of a given bacterium hosting both a *Psuedomonas* plasmid (pMG1, group P2) and *Enterobacteriaceae* plasmids (R6K, R192.7, R64-11, groups X, FII, and I, respectively). In ARO200 some of these plasmids are somewhat less stable than in their usual host, resulting in the loss of resistance determinants in the absence of specific selection. Also, further fragmentation of R-determinant linkages occurs during R^+ ARO200 outcrosses. Twenty percent of the *E. coli* or *P. aeruginosa* R^+ exconjugants from matings with R^+ ARO200 donors showed the loss of at least one R-determinant even though the donors were grown previously on medium containing the corresponding selective antibiotic. Thus, the genetic and metabolic milieu of ARO200 significantly alters the integrity of resident plasmids and their mating behavior. The fragmentation of plasmids not maintained in their usual hosts has been noted before (28). We thought that this apparent instability of R-determinants in ARO200 might facilitate interaction between dissimilar plasmids simultaneously present and thus allow for the dissemination of R-determinants outside of the host range of the parent plasmid. As can be seen in Table 5, this occurred. We observed transfer of R-determinants from *Enterobacteriaceae* plasmids to *P. aeruginosa* as well as transfer of R-determinants from a *Pseudomonas* plasmid to *E. coli*. Thus, in ARO200, diverse narrow host range plasmids interact to an extent that allows subsequent transfer of their R-determinants to unique recipient bacterial strains.

Bacterial strains resistant to antibiotics and showing similar resistance patterns, although incapable of conjugal transfer of these resistances to unrelated bacteria, were observed some time before the relatively recent description of broad host range plasmids, e.g., plasmids RP1, Rs-a. It therefore does not seem likely that broad host range plasmids were principally responsible for the distribution in nature of R-determinants. However, the presence in nature of bacterial strains capable of simultaneously hosting plasmids with limited host range can allow for the distribution of R-determinants after recombination and conjugation. *A. salmonicida*, under some circumstances, is found in an ecological niche that strongly selects for the ascendancy of antibiotic-resistant bacteria. It may, under these conditions, receive R-factors from unrelated donor bacterial strains and thus, following recombination between the heteroplasmids, facilitate redistribution of R-determinants in nature. Heretofore, the potential for the miscegenation of bacterial genes carried on plasmids by broad-host-range plasmids has been obvious and has been shown for RP1 (19). However, another possibility now seems equally feasible, namely, interactions in a common genetic background between genetic vectors of limited host range. It is apparent that some plasmids, in point of fact, have overlapping host ranges when *A. salmonicida* is included in this consideration.

The physical organization and properties of plasmid aggregates presented here is indeterminate. This is also the case for the parent plasmids singly maintained in ARO200. When ARO200 deoxyribonucleic acid (DNA) was radioactively labeled and extracted using conventional techniques for the isolation of plasmid DNA, (3, 11), no supercoiled (covalently closed circular) DNA was obtained. This result corresponds to a previous observation of Hedges et al. for incompatibility group J plasmids (14). Furthermore, when cleared lysates were run directly on neutral sucrose gradients, the sedimentation pattern of labeled DNA resembled that for randomly fragmented host or plasmid DNA sedimenting in the 10*S* to 30*S* regions of the sucrose gradient with no definite and symmetrical DNA peaks apparent (unpublished observation). From this, we surmise that the apparent physical perturbation of plasmid structure may be casually related to the intrinsic properties of ARO200. This aspect is presently being studied using mutant ARO200 strains. Alternatively, but in our view less likely, the physical organization of pMG1, R192.7, and R6K is unique in ARO200, reflecting plasmid-gene expression not observed in other host bacteria.

Several possible mechanisms may be envisaged as the basis for transfer of R-determinants beyond their normal host range. Perhaps most likely would be the transposition of R-determinants between plasmids co-maintained in ARO200 in the manner suggested by Hedges and Jacob (12). These authors have also reported an example of recombination between a large portion of a limited host range plasmid with a broad host range plasmid, R751, followed by transfer of the pseudomonad R-deter-

minants to *E. coli* (13). Either of these possibilities could have occurred at any time during routine maintenance of ARO200 doubles. However, in this instance, recombination led to the formation of a co-integrate recombinant plasmid whose stability was diminished by the incorporation of hetero-antibiotic resistance genes, as evidenced by the behavior described herein. Another possibility may resemble the situation described for transfer of a nontransmissible R-factor by a *Pseudomonas* sex factor wherein the sex factor pilus and mating determinants were apparently utilized to promote independent conjugal transfer of the heteroplasmid (16). In this instance, the plasmid linkage group now transferred beyond its usual host range may have unstable properties reflecting suboptimal plasmid replication-host cell interactions. Thus, survival of the heteroplasmid would depend on continuance of selection for its R-determinants. This presumed tenuous situation also might promote recombination with the mobilizing plasmid in the R^+ exconjugants. We believe that these observations suggest possibilities for extending the host range of plasmid gene functions in the absence of a broad host range R-factor. Further work is being done to distinguish between, on one hand, recombination occurring in heterogeneous donor cell populations resulting in the acquisition of instability for both the parent plasmid and its acquired gene functions and, on the other hand, overcoming transfer barriers by utilization of a permissive plasmid conjugal functions later accompanied by recombination in exconjugants.

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