Resistance Plasmids of *Aeromonads*

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The resistance plasmids of isolates of *Aeromonas hydrophila* and *A. salmonicida* from Japan, France, the UK and Ireland have been characterized. Three classes of plasmids transmissible to *Escherichia coli* were found: plasmids of incompatibility group C, plasmids of incompatibility group U, and plasmids incapable of stable inheritance in *E. coli*, not belonging to any defined incompatibility group.

INTRODUCTION

Aeromonads constitute a well defined genus usually assigned to the family *Vibrionaceae*, although recent studies have suggested that they may be more closely related to the *Enterobacteriaceae* and should, perhaps, be considered as a distinct family (Baumann & Schubert, 1984).

Aoki *et al.* (1971) demonstrated resistance plasmids ('R factors' or R plasmids) in *Aeromonas liquefaciens* (*A. hydrophila*) and transferred them to *Escherichia coli*. Hedges & Datta (1971) showed that these plasmids were of two sorts, half belonging to incompatibility group A, now regarded as part of group C (Sirgel *et al.*, 1981), whilst the others are now regarded as the prototypes of group U (Tschäpe *et al.*, 1981). Mizon *et al.* (1978) studied R factors in *A. hydrophila* isolates from a hospital in Paris. Half were of group C whilst the others were not assigned to any group. A collection of *A. salmonicida* strains from a fish hatchery on the west coast of Ireland was available to us. We decided to characterize the R plasmids from these three sets of aeromonads isolated from very different ecosystems in geographically well separated localities.

METHODS

Bacteria. *Aeromonas* spp. are listed in Table 1.

Techniques for study of plasmids. *Escherichia coli* K12 strains J53 and J62 (and mutants resistant to nalidixic acid or rifampicin) were used as plasmid recipients. Techniques for plasmid transfer and the determination of incompatibility specificity were as described by Datta *et al.* (1971) and Coetzee *et al.* (1972).

Plasmid isolation and molecular weight determination. The techniques of Birnboim & Doly (1979) and Datta *et al.* (1979) were used.

RESULTS

Plasmids transferred from Aeromonas hydrophila

The R plasmids transmissible to *E. coli* fell into three classes: plasmids of incompatibility group U were detected in all three collections, plasmids of group C were transferred from Japanese and French isolates, and both French and Irish aeromonads carried plasmids which were notably unstable in *E. coli*. Representatives of all plasmids transferred are presented in Table 1.

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Table 1. *Plasmids from wild Aeromonas isolates transferred to E. coli K12*

Where indistinguishable plasmids were transferred from more than one *Aeromonas* isolate, only one is included in this list.

Plasmid	Original host	Country of origin	Resistances*	Size (MDal)	Compatibility specificity	Reference
RA1	<i>A. hydrophila</i>	Japan	TcSu	86	C	Hedges & Datta (1971)
RA3	<i>A. hydrophila</i>		SmCmSu	30	U	Hedges & Datta (1971)
pAr32	<i>A. salmonicida</i>		SmCmSu	30	U	Aoki <i>et al.</i> (1979)
R1460†	<i>A. hydrophila</i>		SmTcKmSu	34	U	Mizon <i>et al.</i> (1978)
R1461†	<i>A. hydrophila</i>		SmKmSu	34	U	This paper
R1462†	<i>A. hydrophila</i>	France	Tc	64	New	Mizon <i>et al.</i> (1978)
R1463†	<i>A. hydrophila</i>		SmTcSu	34	U	This paper
Not named‡	<i>A. hydrophila</i>		ApSmTcCmKmGmSu	Not known	C	Mizon <i>et al.</i> (1978)
Not named‡	<i>A. hydrophila</i>		TcCmKm	Not known	C	Mizon <i>et al.</i> (1978)
R1491§	<i>A. salmonicida</i>	UK	SmTcCm	100	C	This paper
pUG1001	<i>A. hydrophila</i>	Ireland	SmTcSuTp	40	U	This paper
pUG1081	<i>A. hydrophila</i>		SmSuTp	38	U	This paper
pUG1031	<i>A. hydrophila</i>		TcCm	60	New	This paper

* Abbreviations: Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin/spectinomycin; Su, sulphonamides; Tc, tetracycline; Tp, trimethoprim.

† These plasmids were transferred from strains of *A. hydrophila* generously supplied by Dr F. Delisle-Mizon (Institut Pasteur, Paris, France). They were not named by Mizon *et al.* (1978) but have been given names in this paper.

‡ These plasmids were described by Mizon *et al.* (1978) and have not been included in our researches. They are typical representatives of the C plasmids described in the paper quoted.

§ R1491 transferred from a strain generously supplied by Dr C. J. Rodgers (MAFF Fish Diseases Laboratory, Weymouth, Dorset, UK).

Plasmids of group U. These plasmids were the only incompatibility group detected in isolates from all three collections. The three sets differed in their characteristic resistance patterns: the Sm and Su resistances were invariable but the other resistances (Cm, in the Japanese, Km and Tc in the French, Tc and Tp amongst the Irish plasmids) were variable within and between populations (Table 1). The former resistances were stable in *E. coli*, and (apart from plasmid-free segregants) no Sm^S or Su^S variants were ever observed, whilst the other resistances were more readily lost (very roughly one sensitive segregant per thousand cells) during growth on drug free media.

These results suggest that, like plasmids of group W (Gorai *et al.*, 1979) and group P (Villarroel *et al.*, 1983), U plasmids have a relatively uniform structure into which transposons are incorporated and spread as the markers they carry are selected. We have no direct evidence of transposability of the Km or Tc markers but the Tp determinant of, for example, pUG1001 readily transposed into the *E. coli* chromosome and appears to resemble closely the well characterized Tn7 (Richards & Nugent, 1979). The sizes of the plasmids fit well with this interpretation (Table 1). The Japanese and French plasmids are all in the 30–34 MDal region and though the Irish plasmids are larger, the presence of a Tn7-like transposon accounts for the increase.

Plasmids of this group have a wide host range and are capable of functioning in many species. It is clear that they are widely distributed in aeromonads, yet the only report of such plasmids in bacteria other than this genus is that of Tschäpe *et al.* (1981), who found several U plasmids in hospital isolates of enteric bacteria from East Germany and Czechoslovakia. The present evidence suggests that the group is characteristic of aeromonads but does occasionally transfer to and establish itself in other genera. Plasmids may acquire some of the transposons present in the gene pools of these bacteria and subsequently ferry these back to the aeromonads. Thus, it may be no coincidence that one of the plasmids described by Tschäpe *et al.* (1981) appears to be similar to pUG1001 (a carrier of the Tn7-like transposon) in molecular weight and resistance pattern. The *EcoRI* restriction nuclease digests were also very similar (G. Brazil, unpublished).

Bradley (1980) showed that transmissible plasmids of enteric bacteria could be divided into two classes, those with flexible pili and those with rigid ones. Members of the latter group (to which U plasmids belong) seem to be opportunistic genetic elements characterized by broad host ranges and constitutive production of sex pili. The majority of these confer sensitivity to the pilus phages of the PRD1 class (Bradley *et al.*, 1982). These phages should be able to cause sudden declines in plasmid-carrying bacterial populations. U plasmids are untypical in not conferring sensitivity to these phages. After a hospital epidemic involving such a phage, there will be an empty ecological niche for an opportunistic plasmid conferring antibiotic resistances, and U plasmids in hospital aeromonads (Mizon *et al.*, 1978) may transfer to enteric bacteria and multiply in these hosts.

The ecology of U plasmids may then be characterized as primarily being typical plasmids of aeromonads with opportunistic spread into other genera when the normal plasmid sets of those bacteria are depleted. The large majority of the U plasmids entering other genera are probably eventually lost (out-competed) but a few, retransferring to aeromonads, may introduce genetic determinants, thus adding to the gene pool of the genus.

Plasmids of group C. R plasmids of this group were found in *A. hydrophila* isolates from both Japan and France. These plasmids have a notably wide natural host range. In view of the possibility that *Aeromonas* may be related to the genus *Vibrio*, it may be worth noting that C plasmids dominate the R plasmid set of *V. cholerae* (Hedges *et al.*, 1977). The plasmids from marine vibrios described by Arai & Aoki (1977) are also members of group C (N. Datta, personal communication). It is, however, notable that another fish pathogen, *Edwardsiella tarda* carries C plasmids (Aoki *et al.*, 1977) and these plasmids may have acquired a role amongst bacteria of diverse lineages in this ecological niche.

Plasmids unstable in E. coli. R plasmids which appear to be stable in the original *A. hydrophila* host but which are highly unstable after transfer to *E. coli* were observed in the French and Irish populations. Representatives (R1462 and pUG220) were transferred from *E. coli* K12 with plasmids representing the known incompatibility groups. In no case did their entry lead to the

elimination of the resident plasmid. Thus, neither plasmid is a member of any defined incompatibility group. As both were extremely unstable it was not possible to test their mutual compatibility. The two plasmids are similar in size and both are decidedly more stable during growth at 30 °C than at 37 °C, (results not shown), and these similarities may indicate relatedness.

Attempts to detect plasmids capable of transfer between strains of *A. hydrophila* (the recipients marked by chromosomal antibiotic resistance mutations), but not into *E. coli*, all failed.

Plasmids transferred from A. salmonicida

A. salmonicida constitutes a well defined species by morphological, taxonomic and molecular criteria (Sakazaki & Balows, 1981; Popoff, 1984) and it has been suggested that it should be elevated to form a separate genus. Nevertheless, the plasmid sets of *A. salmonicida* and *A. hydrophila* are very similar, including plasmids of groups C and U as well as plasmids unstable in *E. coli* (Table 1).

DISCUSSION

It is a general ecological principle that two biological species with exactly similar ecological niches cannot co-exist stably (Gause, 1934). The logic should hold true for plasmid groups, yet it seems that most bacterial species support several distinct plasmid groups; *Aeromonas* supports at least three. One possible explanation is that the populations may not have reached equilibrium. Chemotherapy was started only about 40 years ago and many new drugs have been brought to bear on bacterial populations. The present day plasmid distribution may be a transitional phase: yet, what is known of bacteria in the prechemotherapeutic period indicates a diversity of plasmids comparable with the present day (Datta & Hughes, 1983).

An alternative view is that the different plasmid groups play different ecological roles. The plasmids noted as being unstable in *E. coli* seem to be particularly well adapted to existence in aeromonads and may well be ecologically limited to that genus. Thus, they can evolve towards a more complete integration into the *Aeromonas* genome. On the other hand, C plasmids occur in a very wide range of bacterial families. This type of plasmid cannot adapt so precisely to any one host species but can exploit a huge range of host species (and hence a wide range of physical environments). The role of U plasmids has already been discussed. It may be significant that both C and U plasmids determine pili capable of conferring phage sensitivity (Sirgel *et al.*, 1981; Bradley *et al.*, 1981). Such phages may play significant roles in the ecology of these plasmids.

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