

不和合性群RプラスミドのAeromonas hydrophilaにおける 安定性

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Short Paper

Stability in *Aeromonas hydrophila* of Various Incompatibility Group R Plasmids

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We isolated drug-resistant bacteria from intestinal tracts of six kind of fresh water fish: yamame *Onchorhynchus masou*, rainbow trout *Salmo gairdneri*, ayu *Plecoglossus altivelis*, carp *Cyprinus carpio*, eel *Anguilla japonica* and crucian carp *Carassius auratus* and their culture ponds. The majority were *Aeromonas hydrophila* ranging from 70% (Carp) to 29% (Eel) in the isolates. Drug resistant patterns were examined with tetracycline (TC), chloramphenicol (CM), streptomycin (SM), ampicillin (APC), kanamycin (KM), sulfanilamide (SA), nalidixic acid (NA) and rifampin (RIF). These drugs were purchased commercially. All of these *A. hydrophila* were resistant to APC and some of them were also resistant to SA, TC and other antibiotics. From these resistant strains, 44 R plasmids (29.3%) were isolated¹⁾ and all belonged to incompatibility (Inc) C group when we examined with the previously described method.²⁾ However, Inc C plasmid was isolated only at a frequency of 1 to 2% among *A. hydrophila* strains in stream water without fish cultured pond.¹⁾

To analyze the unexpected results that all plasmids isolated from *A. hydrophila* belonged to Inc C group, 17 standard R plasmids were investigated if they were transferable to *A. hydrophila* and stable in it. Strains carrying the standard R plasmids were provided by Dr. N. Datta³⁾ for the test of plasmid incompatibility and belonged to 17 different incompatibilities. We examined the conjugal transferability of the standard plasmids by using two *A. hydrophila* strains as the recipients (table 1). Both recipients are sensitive to TC, CM, SA, APC, KM and NA but resistant to SM or RIF.

The donors, *Escherichia coli* 21038 *nal-r* or *rif-r*, and recipient strains in the middle exponential phase were mixed at a donor-recipient ratio of 1:1, and incubated

at either 30 or 37°C. After 18 h of incubation, the mixed culture was spread on Mueller-Hinton Agar plates containing drugs¹⁾ selective for transconjugants. Colonies grown on the selective plate were incubated overnight in BHI broth without drugs and spread on a nutrient agar plate. After that, the grown colonies were tested for their antibiotic resistances.²⁾ Among those R plasmids, seven (Inc FII, FV, C, O, P, T, X) were transferable to *A. hydrophila* and only Inc C R-plasmid was quite stable in the new host. These *in vitro* study explained well Inc C plasmids are prevailing in *A. hydrophila*, and these results paralleled the epidemiological studies.¹⁾ Inc C plasmids were characterized by their high transferability at relatively high salt concentration³⁾ and also known to transfer easily to *Vibrio cholerae* or *Vibrio parahaemolyticus*. These plasmids replicate stably in these pathogens.^{2,4)}

Moreover, the Inc C plasmids have wide host range of bacteria including *Pseudomonas* and *Enterobacteriaceae*.⁵⁾ But a clear explanation among the Inc C plasmids has not been made. Therefore, it is necessary to continue the study on spread of R plasmids and bacterial contamination among environmental bacteria.

References

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Table 1. Incompatibility of plasmid and its stability in *A. hydrophila*

| Donor plasmids | Inc group | Drug marker | Transfer frequency ^{*1} | Stability ^{*2} (%) |
|----------------|-----------|-----------------|----------------------------------|-----------------------------|
| R381 | FI | TC | <10 ⁻⁸ | |
| R9-5 | FII | CM | 3.2×10 ⁻⁷ | 40 |
| R124 | FIV | TC | <10 ⁻⁸ | |
| F-lac-tet | FV | TC | 7.1×10 ⁻⁷ | 82 |
| R40a | C | SA, KM, APC | 8.1×10 ⁻⁴ | 100 |
| R27 | H | TC | <10 ⁻⁸ | |
| R144 | Iα | TC, KM | <10 ⁻⁸ | |
| R621a | I | TC | <10 ⁻⁸ | |
| R391 | J | KM | <10 ⁻⁸ | |
| R387 | K | CM, SM | <10 ⁻⁸ | |
| R446b | M | TC, SM | <10 ⁻⁸ | |
| N3 | N | TC, SM, SA | <10 ⁻⁸ | |
| R14 | O | TC, SM, SA, APC | 2.6×10 ⁻⁶ | 50 |
| RP4 | P | TC, KM, APC | 1.0×10 ⁻⁶ | 59 |
| Rts-1 | T | KM | 4.0×10 ⁻⁶ | 63 |
| S-a | W | CM, SM, SA, KM | <10 ⁻⁸ | |
| R6K | X | APC | 3.0×10 ⁻⁵ | 90 |

*1 Recipient. *A. hydrophila* HFM 28 *met pur str-r* or HFM 14 *his try rif-r*.

*2 Parentheses expressed as a percentage.

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