

Transferable Drug Resistance Plasmids in Fish-Pathogenic Bacteria

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ABSTRACT

Chemotherapeutic agents have been developed for treating bacterial infections and have been widely used for cultured fish for the last 30 years in Japan. The extensive use of chemotherapeutants has resulted in an increase in the occurrence of drug resistance in fish-pathogenic bacteria and also in the bacterial flora of the intestinal tract of cultured fish. The kinds of chemotherapeutants used are correlated with the occurrence of the corresponding drug-resistant genes in fish-pathogenic bacteria. Almost all multiple-drug resistant strains are carried on the transferable R plasmid, although resistance in fish pathogens to nitrofurans and pyridonecarboxylic acids is associated with a chromosomal gene. The DNA sequences of R plasmids generally differ depending on the species of fish pathogen. Exceptions are the R plasmids of *Aeromonas hydrophila* and *A. salmonicida*, which have the same resistance markers as chloramphenicol, streptomycin, and sulfonamides (SA); and the R plasmids of *A. hydrophila* and *Edwardsiella tarda*, which have the same resistance markers as SA and tetracycline. The fish pathogens *A. hydrophila*, *A. salmonicida*, *E. tarda*, *Enterococcus seriolicida*, *Pasteurella piscicida*, and *Vibrio anguillarum* are all widely distributed in fish farms in various areas, and within each species the R plasmid has an identical DNA sequence. The chloramphenicol resistance (*cat*) gene of the R plasmid from Gram-negative bacteria was classified into CAT I, II, III, and IV according to the DNA sequence. The *cat* gene of *P. piscicida* was classified as CAT I, those of *A. salmonicida* and *E. tarda* were classified as CAT II, and that of *V. anguillarum* was classified as CAT II or IV, depending on the time the strains were isolated. The tetracycline-resistance determinants (Tet), which occur in six classes (Tet A through Tet G), were class D in the R plasmids obtained from strains of *V. anguillarum* that were isolated from 1989 to 1991. The Tet for strains of *V. anguillarum* isolated from 1973 to 1977 was classified as Tet B, while for strains isolated from 1980 to 1983 it was classified as Tet G.

INTRODUCTION

Intensive fish culture has resulted in the massive use of chemotherapeutic agents for the treatment of bacterial infectious diseases. The most common chemotherapeutants in use in fish farms in Japan are amoxicillin, ampicillin, bicozamycin, florfenicol, lincomycin, macrolide antibiotics, novobiocin, pyridonecarboxylic acids, sodium nifurstyrenate, sulfonamides, the sulfonamide:ormetoprim complex, tetracycline derivatives, and thiamphenicol (Aoki 1992a, Kitao *et al.* 1992).

Drug-resistant Gram-negative bacteria carrying the transferable R plasmid were isolated with high frequency from the intestinal tracts of cultured eel (*Anguilla* spp.) (Aoki and Watanabe 1973a), carp (*Cyprinus carpio*) (Aoki 1974), amago salmon (*Oncorhynchus rhodurus*) (Aoki *et al.* 1972), ayu (*Plecoglossus altivelis*) (Aoki 1975a, Aoki *et al.* 1980), and yellowtail (*Seriola quinqueradiata*) (Aoki *et al.* 1973) in which chemotherapeutants had often been used for treatment. On the other hand, drug-resistant bacteria were isolated at low frequencies from the intestinal tracts of wild ayu,

as well as from cultured ayu that were not administered chemotherapeutants (Aoki 1975a, Aoki *et al.* 1980).

Various drug-resistant bacteria were isolated with varying frequency from the water of ponds used for culturing eel (Aoki and Watanabe 1973a), carp (Aoki 1974), ayu (Aoki 1975a), and rainbow trout (Aoki and Watanabe 1973b).

The use of chemotherapeutants in aquaculture has been associated with an increased occurrence of drug-resistant fish-pathogenic bacteria, including *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *Enterococcus seriolicida*, *Pasteurella piscicida*, and *Vibrio anguillarum* (Aoki 1988, 1992a, b; Aoki *et al.* 1990).

When donor and recipient cells were cultured together in a test tube, the R plasmid could be easily transferred from donor cells of drug-resistant strains of fish pathogens to recipient cells of a strain of *Escherichia coli*. Transfer of R plasmids was also observed in sterilized sea water (Goodman *et al.* 1993), sediments (Sandaa and Enger 1994), and pond water used for fish culture (Aoki 1974); however, *in vitro* transfer of R plasmids was rare in the intestinal tracts of carp (Aoki 1975b) and mouse (Wiedemann 1972).

Multiple drug-resistant strains of fish pathogens that carry the transferable R plasmid were widely distributed in fish farms in various areas (Aoki 1992a, b). The detected R plasmids from Gram-negative fish pathogens were encoded with resistance to ampicillin, chloramphenicol, florfenicol, kanamycin, ormetoprim, sulfonamides, and/or tetracycline. Drug-resistant markers of R plasmids depend on the origin of each fish pathogen. The kind of chemotherapeutant used was correlated with the appearance of the corresponding drug-resistant marker, except for kanamycin (KM) and streptomycin (SM) of *P. piscicida* and *V. anguillarum*. KM and SM have never been used to treat any bacterial infectious disease in Japanese fish farms (Aoki 1988, 1992a, b). Gram-negative pathogenic bacteria of fish that also have chromosomal genes that provide resistance to pyridonecarboxylic acids and nitrofurans derivatives were isolated frequently in fish farms (Aoki 1988, 1992a, b).

Drug-resistant strains of the Gram-positive *Enterococcus seriolicida* have resistance to various combinations of CP, erythromycin (Mls), lincomycin (LIM), and TC. These resistant strains were classified as having either intermediate- or high-level resistance to CP, LIM, Mls, or TC. Transferable R plasmids were detected in high-level resistant strains with resistance to CP, LIM, Mls, and/or TC. Intermediate-level resistance determinants were not transferred (Aoki *et al.* 1990).

The DNA sequences of R plasmids from *A. hydrophila*, *A. salmonicida*, *E. tarda*, *E. seriolicida*, *P. piscicida*, and *V. anguillarum* differed from each other (Aoki 1988, 1992b). R plasmids of *A. hydrophila* and *A. salmonicida* have the same markers as CP, SA and SM, and the R plasmids of *A. hydrophila* and *E. tarda* also have the same resistance markers as SA and TC (Akashi and Aoki 1986, Aoki *et al.* 1986). The R plasmids detected from each fish pathogen, which were isolated in different areas but in the same year, had identical DNA sequences.

The chloramphenicol resistance (*cat*) gene of the R plasmid from Gram-negative bacteria was classified into CAT I, II, III and IV according to the DNA sequence. The *cat* gene of the R plasmid detected in *P. piscicida* was classified as CAT I and those from *A. salmonicida* and *E. tarda* were classified as CAT II. The *cat* gene of the R plasmid from *V. anguillarum* was classified as CAT II for strains isolated between 1973 and 1977 and as CAT IV for strains isolated between 1989 to 1991 (Aoki 1988, Kim and Aoki 1993).

Six classes of tetracycline resistance determinant (Tet) of the R plasmids from Gram-negative bacteria (Tet A through G) were recognized. The Tet in R plasmids from *A. hydrophila*, *E. tarda*,

P. piscicida and *V. anguillarum* (isolated from 1989 to 1991) was classified as Tet D (Aoki and Takahashi 1987), which is very common in fish pathogens. The Tet of R plasmids from *V. anguillarum* isolated from 1973 to 1977 was Tet B, while that from those isolated from 1980 to 1991 was Tet G (Aoki 1988).

REFERENCES

- Akashi A, Aoki T. 1986. Characterization of transferable R plasmids from *Aeromonas hydrophila*. Bull. Jpn. Soc. Sci. Fish. 52:649-655.
- Aoki T. 1974. Studies of drug-resistant bacteria isolated from water of carp-ponds and intestinal tracts of carp. Bull. Jpn. Soc. Sci. Fish. 40:247-254. (in Japanese).
- Aoki T. 1975a. Effects of chemotherapeutics on bacterial ecology in the water of ponds and the intestinal tracts of cultured fish, ayu (*Plecoglossus altivelis*). Jpn. J. Microbiol. 19:7-12.
- Aoki T. 1975b. Transfer of R factor in the intestinal tracts of fish and sterilized water of cultured pond. Fish Pathol. 9:167-173. (in Japanese).
- Aoki T. 1988. Drug-resistant plasmids from fish pathogens. Microbiol. Sci. 5:219-223.
- Aoki T. 1992a. Chemotherapy and drug resistance in fish farms in Japan. In: Shariff M, Subasinghe RP, Arthur JR. eds. Diseases in Asian Aquaculture I. p. 519-529. Fish Health Section, Asian Fisheries Society, Manila.
- Aoki T. 1992b. Present and future problems concerning the development of resistance in aquaculture. In: Chemotherapy in Aquaculture: from Theory to Reality. p. 254-262. Office International des Epizooties, Paris.
- Aoki T, Akashi A, Sakaguchi T. 1986. Phylogenetic relationships of transferable R plasmids from *Edwardsiella tarda*. Bull. Jpn. Soc. Sci. Fish. 52:1173-1179.
- Aoki T, Egusa S, Watanabe T. 1973. Detection of R+ bacteria in cultured marine fish, yellowtails (*Seriola quinqueradiata*). Jpn. J. Microbiol. 17:7-12.
- Aoki T, Egusa S, Yada C, Watanabe T. 1972. Studies of drug resistance and R factors in bacteria from pond-cultured salmonids. I. Amago (*Oncorhynchus rhodurus macrostomus*) and yamame (*Oncorhynchus masou ishikawae*) Jpn. J. Microbiol. 16:233-238.
- Aoki T, Jo Y, Egusa S. 1980. Frequent occurrence of drug resistant bacteria in ayu (*Plecoglossus altivelis*) culture. Fish Pathol. 15:1-6. (in Japanese).
- Aoki T, Takahashi A. 1987. Class D tetracycline resistance determinants of R plasmids in fish pathogens *Aeromonas hydrophila*, *Edwardsiella tarda*, and *Pasteurella piscicida*. Agents Chemother. 31:1278-1280.
- Aoki T, Takami K, Kitao T. 1990. Drug resistance in a non-haemolytic *Streptococcus* sp. isolated from cultured yellowtail *Seriola quinqueradiata*. Dis. Aquat. Org. 8:171-177.
- Aoki T, Watanabe T. 1973a. Studies of drug-resistant bacteria isolated from eel-pond-water and intestinal tracts of the eel (*Anguilla japonica* and *Anguilla anguilla*). Bull. Jpn. Soc. Sci. Fish. 39:121-130. (in Japanese).
- Aoki T, Watanabe T. 1973b. Studies of drug resistance and R factors in bacteria from pond-cultured salmonids. II. Rainbow trout (*Salmo gairdneri* f. *irideus*). Fish Pathol. 8:83-90.
- Goodman AE, Hild E, Marshall KC, Hermansson M. 1993. Conjugative plasmid transfer between bacteria under simulated marine oligotrophic conditions. Appl. Environ. Microbiol. 59:1035-1040.
- Kim EH, Aoki T. 1993. The structure of the chloramphenicol resistance gene on a transferable R plasmid from the fish pathogen, *Pasteurella piscicida*. Microbiol. Immunol. 37:705-712.
- Kitao T, Yoshida T, Kusuda R, Matsuoka T, Nakano S, Okada M, Oshima T. 1992. *In vitro* antibacterial activity of bicozamycin against *Pasteurella piscicida*. Fish. Pathol. 27:109-113.
- Sandaa RA, Enger O. 1994. Transfer in marine sediments of the naturally occurring plasmid pRAS1 encoding multiple antibiotic resistance. Appl. Environ. Microbiol. 60:4234-4238.
- Wiedemann B. 1972. Resistance transfer *in vivo* and its inhibition. In: Bacterial Plasmids and Antibiotic Resistance. p. 75-90. Springer-Verlag, Berlin.