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Identification and Cloning of a Tetracycline Resistance Gene from the Fish Pathogen *Vibrio salmonicida*

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***Vibrio salmonicida* is the causative agent of cold-water vibriosis in farmed Atlantic salmon. We cloned a 6.9-kb *Pst*I fragment from the 170-MDa plasmid (pRVS1) containing a tetracycline resistance determinant. A subcloned 1.96-kb *Hin*dIII fragment was found to mediate the tetracycline resistance. The 2.5-kb *Cla*I-*Pvu*I fragment carrying the Tet E determinant from *Escherichia coli* hybridized under stringent conditions with the cloned 1.96-kb *Hin*dIII fragment from *V. salmonicida*. The 1.96-kb *Hin*dIII fragment codes for a protein of 26.5 kDa which represents a candidate for the structural TetA protein of the class E determinant. Deletion of a 0.28-kb *Bal*I fragment from the middle of the *Hin*dIII fragment resulted in the loss of tetracycline resistance. We were able to show that when *E. coli* carries the cloned 6.9-kb *Pst*I fragment, expression of tetracycline resistance is regulated by the concentration of tetracycline in the medium. In contrast, tetracycline resistance was constitutively expressed in the *E. coli* isolate carrying the 1.96-kb *Hin*dIII fragment. The tetracycline resistance gene isolated from the 170-MDa R plasmid of the marine fish pathogen *V. salmonicida* was characterized and shown to be a class E determinant.**

Vibrio salmonicida is the causative agent of cold-water vibriosis or Hitra disease, which is primarily reported in farmed Norwegian salmonids (11, 12) and, lately, among farmed cod in Norway (28). The bacterium has been isolated from an outbreak of cold-water vibriosis among farmed salmon in the Shetland Islands (5) and also from several outbreaks in the Faroe Islands (8). *V. salmonicida* has also been isolated from diseased farmed salmon in Canada (New Brunswick and Nova Scotia) (32).

In severe cases of cold-water vibriosis, 50 to 90% of the fish are lost if there is no treatment (14). Oxytetracycline is the antimicrobial agent most frequently used in Norway to treat fish with cold-water vibriosis. In 1985, the first isolates of *V. salmonicida* resistant to tetracyclines were observed (14). In the period from July 1986 to July 1987, 56% of 250 isolates from outbreaks of cold-water vibriosis, at numerous locations along the coast of Norway, were found to be resistant to tetracycline, as determined by the disk diffusion method (29).

After more than 6 years of treatment of cold-water vibriosis with oxytetracycline without any detectable resistance to the drug in isolates of *V. salmonicida*, tetracycline-resistant (Tc^r) isolates were discovered along the entire coast of Norway within a period of a few months (13). Tetracycline-resistant *V. salmonicida* represents the first major antimicrobial resistance problem among fish pathogens in the Norwegian fish farming industry. With the limited geographical occurrence of disease caused by *V. salmonicida*, we were interested in characterizing the Tc^r determinant at the molecular level to see whether it was similar to known, characterized gram-negative Tet determinants (Tet A to E) (18). This was important because of the growing public concern in Norway about the use of antimicrobial agents in the fish farming industry and the possibility of transfer of drug resistance determinants from aquatic bacteria to human pathogens, especially since the Japanese have found Tc^r pathogens in their farmed fish, which carry Tet determinants that have been associated with human pathogenic bacteria (1). In this report, we show that *V. salmonicida* has a Tc^r determinant of the class E type which has been commonly found in *Aeromonas hydrophila* from channel catfish farmed in the southern United States (10).

MATERIALS AND METHODS

Bacteria, plasmids, media, and nomenclature. *V. salmonicida* 1099/87 L1R was isolated in 1987 from a diseased salmon. The uninduced MIC of tetracycline for 1099/87 L1R (20 μg/ml) was typical of resistant isolates. MICs were determined as described elsewhere (16). *Escherichia coli* HB101 Str^r (streptomycin resistant) (4) was used as a recipient in transformation experiments. Plasmid pACYC177, with ampicillin resistance (Ap^r) and kanamycin resistance (Km^r) as genetic markers (6), was used as the vector for cloning experiments.

V. salmonicida was grown aerobically at 15°C in Luria broth supplemented with 1.5% NaCl under constant shaking or on Luria agar supplemented with 1.5% NaCl (26). This agar medium was also used for susceptibility testing by the disk diffusion method (2). *E. coli* was grown aerobically at 37°C in Luria medium supplemented with 20 μg of tetracycline per ml when tetracycline was used for selection.

The nomenclature used for tetracycline resistance determinants in this study follows the recommendation of Levy et al. (20).

Induction of Tc^r by exposure to tetracycline. Induction was performed by adding 1 μg of tetracycline per ml to logarithmic-phase cultures for 45 min prior to challenge with 10 μg of tetracycline per ml for *V. salmonicida* or 20 μg/ml for *E. coli* carrying the cloned *V. salmonicida* Tet gene. Experiments were performed as described previously (19), with *V. salmonicida* incubated at 15°C and *E. coli* incubated at 37°C. Induction of the in vitro transcription-translation reactions was performed with 1.5 μg of tetracycline per ml.

Plasmid and total DNA isolation. Both the Birnboim-Doly

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and sodium dodecyl sulfate (SDS) lysis methods were used to isolate plasmid DNA from *V. salmonicida*, whereas plasmids from *E. coli* were isolated by the Birnboim-Doly method (21). All plasmids were purified in cesium chloride-ethidium bromide density gradients (21). Plasmid DNA was visualized after electrophoresis in 1% agarose gels. Estimates of plasmid copy number for *V. salmonicida* plasmids were made visually from photos of agarose gels stained with ethidium bromide and photographed under UV light exposure.

Whole-cell DNA from *V. salmonicida* was isolated by using lysozyme and Sarkosyl (15) and was then purified in cesium chloride gradients. Restriction-digested whole-cell DNA was separated by electrophoresis in 0.7% agarose gels.

Restriction endonuclease analysis and cloning experiments. Restriction endonuclease digestions were performed as recommended by the manufacturer (Bethesda Research Laboratories, Inc., Gaithersburg, Md.).

Plasmid DNA prepared from *V. salmonicida* was digested with *Pst*I and ligated to the *Pst*I-digested vector pACYC177 (Ap^r Km^r), and the recombinant DNA was transformed into *E. coli* HB101 (7). Prospective clones were screened on agar medium containing tetracycline (5 μg/ml) and kanamycin (50 μg/ml).

Subcloning was carried out by digestion of the recombinant plasmid with *Hin*dIII and ligation to *Hin*dIII-digested pACYC177 DNA. Subclones were screened on medium with tetracycline (5 μg/ml) and ampicillin (100 μg/ml). Restriction enzyme-digested DNAs of recombinant plasmids were separated by electrophoresis on 0.7% agarose gels.

In vitro mutagenesis. A 0.28-kb *Bal*I fragment within the subcloned *Hin*dIII fragment containing the Tc^r gene was deleted by *Bal*I digestion and religation. A stop linker (*Xba*I, amber stop, 14-mer; Pharmacia, Uppsala, Sweden) was introduced into the new *Bal*I site within the *Hin*dIII fragment. The localization of the stop linker was verified by agarose electrophoresis of *Xba*I-digested recombinant DNA. The internal *Xba*I site of the stop linker was unique to the cloned DNA fragment.

Polypeptide analysis. A prokaryotic DNA-directed transcription-translation kit (Amersham International plc, Amersham, England) was used for polypeptide analysis of selected clones. ^{35}S-labeled methionine was used to label the proteins produced in vitro. The radiolabeled proteins were denatured and visualized after electrophoresis in 15% SDS-polyacrylamide gels. The gels were dried under heat and vacuum before exposure to Kodak XRP or XAR film at −70°C for 48 h.

DNA-DNA hybridizations. Colony and Southern blots of agarose gels were prepared as described previously (21, 30). ^{32}P-labeled probes were hybridized to colony and Southern blots under stringent conditions. Dot blots or Southern blots were prepared on nitrocellulose (Schleicher & Schuell, Dassel, Germany) and hybridized overnight at 42°C under stringent conditions of 50% (vol/vol) formamide, 0.1% (wt/vol) polyvinylpyrrolidone, 0.1% (wt/vol) albumin, 0.1% (wt/vol) Ficoll, 0.1% (wt/vol) SDS, 0.05 M monobasic sodium phosphate (pH 7.4), 0.005 M EDTA, 0.76 M NaCl, and 100 μg of boiled calf thymus DNA per ml. The filters were then washed three times for 10 min each time at 52°C in 0.1% SDS–0.015 M NaCl–0.0015 M sodium citrate and then three times for 10 min each time at 52°C in 0.015 M NaCl–0.0015 M sodium citrate, as described previously (21). Positive and negative controls were included in each set. By this procedure, none of the probes cross-hybridized. The following plasmids were labeled with ^{32}P and used as probes: pSL18 (Tet A) (9), pRT11 (Tet B) (23), pBR322 (Tet C) (3), pSL106 (Tet D) (24, 25), and pSL1504 (Tet E) (22). The 6.9- and 1.96-kb fragments from *V. salmonicida* and the 2.5-kb *Cla*I-*Pvu*I fragment from pSL1504 (Tet E) were also labeled and used as probes. The DNA was labeled by the random primer labeling technique (Bethesda Research Laboratories) or by nick translation (27).

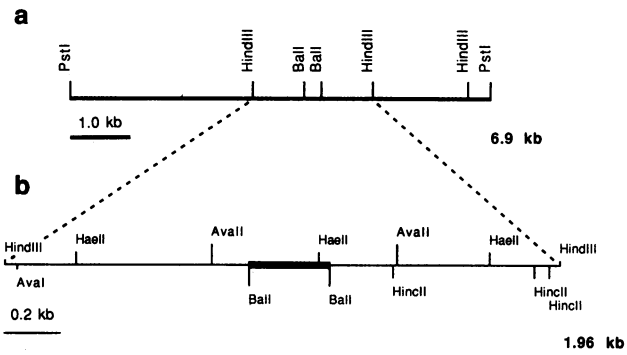

FIG. 1. Map of DNA fragments from *V. salmonicida* 1099/87 L1R cloned within pACYC177 to generate pHS1 (a) and pHS2 (b). The 0.28-kb *Bal*I fragment, which was deleted from pHS2 for the in vitro mutagenesis experiment, is indicated by a bold line.

RESULTS

Cloning experiments. *V. salmonicida* 1099/87 L1R was resistant to tetracycline (MIC, 20 μg/ml, uninduced) and was found to contain a large (170-MDa) plasmid. This plasmid was present in a very low copy number, as determined with ethidium bromide-stained agarose gels, and was not detected in tetracycline-susceptible (Tc^s) strains. A 21-MDa plasmid has been found in all of the several hundred strains of *V. salmonicida* screened for plasmid content, regardless of their susceptibilities to tetracycline (28, 29) and was also seen in strain 1099/87 L1R. A *Pst*I restriction digest was performed on the 21- and 170-MDa plasmids; the DNA was then ligated to the *Pst*I-digested vector, pACYC177 and was transformed into *E. coli* HB101. Transformants were selected on tetracycline (5 μg/ml) and kanamycin (50 μg/ml) and were shown to contain the vector with a 6.9-kb fragment insert in the *Pst*I site (pHS1) (Fig. 1). The cloned fragment hybridized with the 170-MDa plasmid but not with the 21-MDa plasmid from *V. salmonicida* 1099/87 L1R (data not shown). A restriction map of the recombinant plasmid (pHS1) was made (Fig. 1). The tetracycline resistance gene was subcloned into pACYC177 by using *Hin*dIII. Transformants were selected on tetracycline (5 μg/ml) and ampicillin (100 μg/ml). The resulting transformants contained pACYC177 with a 1.96-kb fragment insert in the *Hin*dIII site (pHS2) (Fig. 1).

Induction of the Tc^r gene and MICs. The Tc^r gene from *V. salmonicida* was inducible both in its native host (data not shown) and when it was cloned into *E. coli* (Fig. 2). The induced MIC of tetracycline for *V. salmonicida* was 30 μg/ml, whereas the induced MIC for *E. coli* containing pHS1 with the 6.9-kb fragment was 150 μg/ml, and for *E. coli* containing pHS2 with the 1.96-kb fragment it was 170 μg/ml. The expression of tetracycline resistance from the 1.96-kb fragment in *E. coli* was constitutive, in contrast to the inducible nature of the gene in the 6.9-kb fragment (Fig. 2).

Polypeptide analysis. The in vitro transcription-translation experiments demonstrated that pHS1 with the 6.9-kb *Pst*I

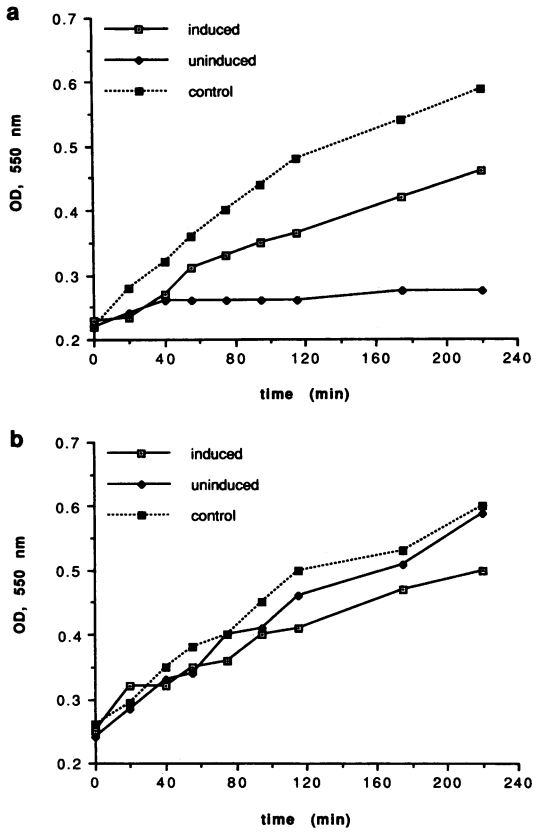

FIG. 2. Expression of tetracycline resistance in *E. coli* HB101 regulated by tetracycline. Growth of logarithmic-phase cultures of HB101 containing pHS1 (a) and pHS2 (b) was measured in the presence of 1 μg of tetracycline per ml for 45 min (induced) or without tetracycline (uninduced) before the cultures were challenged with 20 μg of tetracycline per ml at time zero. The control culture was not exposed to tetracycline. OD, optical density.

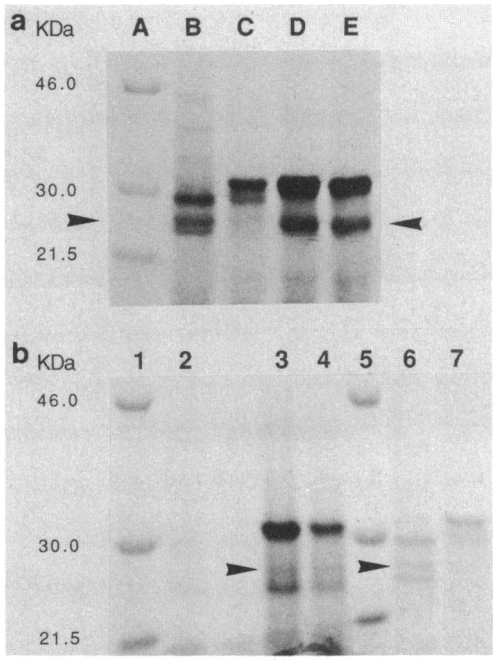

FIG. 3. (a) Fluorograph of an SDS-polyacrylamide gel showing polypeptides labeled with [^{35}S]methionine in an in vitro transcription-translation experiment. The system was induced with 1.5 μg of tetracycline per ml. Lane A, molecular weight markers; lane B, pHS1; lane C, pACYC177; lane D, pHS2; lane E, pHS2 with deletion of the 0.28-kb *Bal*I fragment. Lanes B, D, and E had a 26.5-kDa polypeptide (arrowheads), a candidate for the TetA protein of the class E determinant. (b) Fluorograph showing polypeptides produced with and without induction. Lane 1, molecular weight markers; lane 2, pHS1, uninduced; lane 3, pHS2, uninduced; lane 4, pHS2, induced; lane 5, weight markers; lane 6, pHS1, induced; lane 7, pACYC177. Lanes 3, 4, and 6 had a 26.5-kDa protein (arrowheads). Lane 3 and 4 had an extra band which was lighter than the 26.5-kDa protein and which could be a degradation product of the intact TetA protein.

fragment produced an inducible protein of 26.5 kDa (Fig. 3). pHS2 with the 1.96-kb *Hin*dIII fragment produced a constitutively expressed 26.5-kDa protein (Fig. 3). The 1.96-kb *Hin*dIII fragment with a deletion of the 280-bp *Bal*I fragment still produced a protein of 26.5 kDa, with or without a stop linker in the new *Bal*I site (Fig. 3), but Tc^r was lost. This protein was constitutively expressed (data not shown).

Hybridization studies. Probes of Tet A, B, C, D, and E were hybridized with DNAs from tetracycline-susceptible and -resistant strains of *V. salmonicida*. The probes did not hybridize to any of the susceptible strains, while the Tet E probe hybridized with the Tc^r strains (Fig. 4).

The 2.5-kb *Cla*I-*Pvu*I fragment from pSL1504 (Tet E) was labeled and hybridized against *Hin*dIII and *Eco*RI digests of total DNA from Tc^r *V. salmonicida* strains. A 1.9-kb *Hin*dIII fragment and a 20.5-kb *Eco*RI fragment hybridized with the probe. This probe also hybridized with both the 6.9- and 1.96-kb fragments of pHS1 and pHS2 (data not shown). According to Tovar et al. (31), the pSL1504 plasmid contains most of the structural gene encoding the TetA(E) protein except for a fragment encoding the amino-terminal end of the protein. No part of the regulatory region or the repressor gene of the Tet E determinant is present in this plasmid.

DISCUSSION

Tetracyclines have been used for the treatment of bacterial infections in humans and animals for a number of years and have been associated with the increased occurrence of bacteria that are resistant to antimicrobial agents (17). In this study, we demonstrated that a Tc^r gene was on a 1.96-kb *Hin*dIII fragment which is located within the 170-MDa plasmid (pRVS1) found in *V. salmonicida*. This represents the first reported antimicrobial resistance determinant in this marine bacterium.

The hybridization experiments demonstrated that this determinant has homology, under conditions of high stringency (which detects ≥ 80% homology), with the previously described Tet E structural gene, denoted by *tetA*(E), which was found in a single isolate of *E. coli* from a human (22) and strains of *Aeromonas hydrophila* from channel catfish (*Ictalurus punctatus*) (10).

Hybridization of the labeled 2.5-kb *Cla*I-*Pst*I fragment from pSL1504 containing the *tetA*(E) gene with total DNA from *V. salmonicida* digested with *Hin*dIII and *Eco*RI resulted in one hybridizing fragment in each digest. The size of the homologous *Hin*dIII fragment was identical to that of the cloned fragment in pHS2 (1.96 kb), while the *Eco*RI fragment was 20.5 kb. This agrees with the restriction map of the

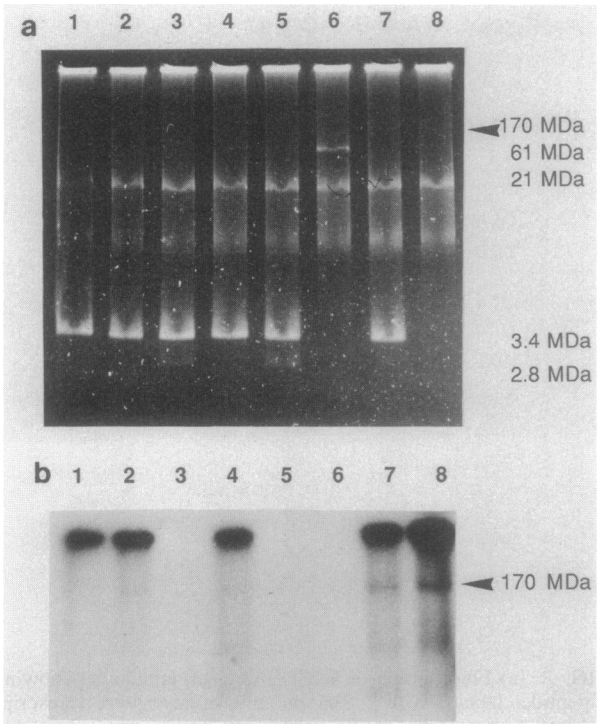

FIG. 4. Hybridization experiment in which pSL1504 with the Tet E determinant was used as a probe against plasmid DNA of tetracycline-resistant and -susceptible *V. salmonicida*. The 170-MDa plasmid is marked with an arrowhead. (a) Plasmid DNA of Tc^r strains (lanes 1, 2, 4, 7, and 8) and Tc^s strains (lanes 3, 5, and 6). Plasmid sizes are in megadaltons. (b) X ray of a Southern blot of the gel shown in panel a in which pSL1504 was used as a probe.

cloned fragment of 6.9 kb in pHS1, which has a 1.96-kb *Hin*dIII fragment but no *Eco*RI site. These data support the hypothesis that the cloned Tet determinant is located on the large (170-MDa) plasmid pRVS1 of *V. salmonicida*.

The restriction map of the Tet E determinant from pRVS1 appears to be unrelated to the published map of the Tet E determinant isolated from *E. coli* (22). The differences between the two Tet E determinants should be clarified once the *V. salmonicida* gene is sequenced. That work is in progress.

The *E. coli* isolate carrying the 6.9-kb fragment showed inducible resistance to tetracycline, while the strain carrying the 1.96-kb fragment encoded constitutive tetracycline resistance. This suggests that the 6.9-kb fragment carries a repressor gene which is absent from the smaller fragment. The presence of a repressor gene has been described previously for the Tet E determinant isolated in *E. coli* (31).

When a 280-bp *Bal*I fragment was deleted from the 1.96-kb *Hin*dIII fragment, the tetracycline resistance was lost but a protein of 26.5 kDa, like that from the intact gene, was still produced. This was also the case if a stop linker was introduced into the new *Bal*I site. An explanation of this may be that the intact structural TetA(E) protein is degraded in the in vitro system by being split into two or more polypeptides close to where the deletion was made. In some protein gels there are two weaker bands instead of a single strong one from the strains with the constitutively expressed gene. One of the bands was 26.5 kDa; the other one was smaller (Fig. 3).

Conjugal transfer of Tc^r from *V. salmonicida* to other bacteria, including *V. salmonicida*, *Vibrio anguillarum* (fish pathogens), and *E. coli*, was attempted but proved to be unsuccessful (data not shown). Similar results were reported with *Aeromonas hydrophila* donors, containing plasmids with the Tet E determinant, mated with *E. coli* recipients (10). In contrast, R plasmids from the bacterial fish pathogens *V. anguillarum* and *Pasteurella piscicida* isolated in Japan have been transferred by conjugation to *E. coli* (1). These R plasmids contain genes that mediate resistance to up to five of the following antimicrobial drugs on a single plasmid: tetracycline, cloramphenicol, sulfonamides, kanamycin, streptomycin, or ampicillin (1).

In conclusion, our results indicate the presence of the Tet E determinant in Tc^r *V. salmonicida* isolated in Norway. The presence of this Tet E determinant is similar to that of the Tet E determinant in Tc^r *A. hydrophila* strains isolated from channel catfish in the southern United States.

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REFERENCES

1. **Aoki, T.** 1988. Drug-resistant plasmids from fish pathogens. Microbiol. Sci. **5**:219–223.
2. **Barry, A. L., and C. Thornsberry.** 1985. Susceptibility tests: diffusion test procedures, p. 978–987. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
3. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
5. **Bruno, D. W., T. S. Hastings, A. E. Ellis, and R. Wootten.** 1985. Outbreak of a cold-water vibriosis in Atlantic salmon in Scotland. Bull. Eur. Assoc. Fish Pathol. **5**:62–63.
6. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the p15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
7. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
8. **Dalsgaard, I., O. Jürgens, and A. Mortensen.** 1988. *Vibrio salmonicida* isolated from farmed Atlantic salmon in the Faroe Islands. Bull. Eur. Assoc. Fish Pathol. **8**:53–54.
9. **DeFlaun, M. F., and S. B. Levy.** 1989. Genes and their varied hosts, p. 1–32. *In* S. B. Levy and R. V. Miller (ed.), Gene transfer in the environment. McGraw-Hill Publishing Co., New York.
10. **DePaola, A., P. A. Flynn, R. M. McPhearson, and S. B. Levy.** 1988. Phenotypic and genotypic characterization of tetracycline- and oxytetracycline-resistant *Aeromonas hydrophila* from cultured channel catfish (*Ictalurus punctatus*) and their environments. Appl. Environ. Microbiol. **54**:1861–1863.
11. **Egidius, E., K. Andersen, E. Clausen, and J. Raa.** 1981. Cold-water vibriosis or "Hitra disease" in Norwegian salmonid farming. J. Fish Dis. **4**:353–354.
12. **Egidius, E., R. Wiik, K. Andersen, K. A. Hoff, and B. Hjeltnes.** 1986. *Vibrio salmonicida* sp. nov., a new fish pathogen. Int. J. Syst. Bacteriol. **36**:518–520.
13. **Håstein, T. (National Veterinary Institute, Oslo, Norway).** 1991. Personal communication.

14. **Hjeltnes, B., K. Andersen, and E. Egidius.** 1987. Multiple antibiotic resistance in *Vibrio salmonicida*. Bull. Eur. Assoc. Fish Pathol. **7:**85.
15. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33:**933–938.
16. **Jones, R. N., A. L. Barry, T. L. Gavan, and J. A. Washington II.** 1985. Susceptibility tests: microdilution and macrodilution broth procedures, p. 972–977. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
17. **Levy, S. B.** 1984. Resistance to the tetracyclines, p. 191–240. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., Orlando, Fla.
18. **Levy, S. B.** 1988. Tetracycline resistance determinants are widespread. ASM News **54:**418–421.
19. **Levy, S. B., A. Buu-Hoi, and B. Marshall.** 1984. Transposon Tn*10*-like tetracycline resistance determinants in *Haemophilus parainfluenzae*. J. Bacteriol. **160:**87–94.
20. **Levy, S. B., L. M. McMurry, V. Burdett, P. Courvalin, W. Hillen, M. C. Roberts, and D. E. Taylor.** 1989. Nomenclature for tetracycline resistance determinants. Antimicrob. Agents Chemother. **33:**1373–1374.
21. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Marshall, B., S. Morrissey, P. Flynn, and S. B. Levy.** 1986. A new tetracycline-resistance determinant, class E, isolated from *Enterobacteriaceae*. Gene **50:**111–117.
23. **Marshall, B., M. Roberts, A. Smith, and S. B. Levy.** 1984. Homogeneity of transferable tetracycline-resistance determinants in *Haemophilus* species. J. Infect. Dis. **149:**1028–1029.
24. **Marshall, B., C. Tachibana, and S. B. Levy.** 1983. Frequency of tetracycline resistance determinant classes among lactose-fermenting coliforms. Antimicrob. Agents Chemother. **24:**835–840.
25. **Mendez, B., C. Tachibana, and S. B. Levy.** 1980. Heterogeneity of tetracycline resistance determinants. Plasmid **3:**99–108.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Rigby, P. W., J. M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113:**237–251.
28. **Sørum, H., A. B. Hvaal, M. Heum, F. L. Daae, and R. Wiik.** 1990. Plasmid profiling of *Vibrio salmonicida* for epidemiological studies of cold-water vibriosis in Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). Appl. Environ. Microbiol. **56:**1033–1037.
29. **Sørum, H., T. T. Poppe, and Ø. Olsvik.** 1988. Plasmids in *Vibrio salmonicida* isolated from salmonids with Hemorrhagic syndrome (Hitra disease). J. Clin. Microbiol. **26:**1679–1683.
30. **Southern, E.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.
31. **Tovar, K., A. Ernst, and W. Hillen.** 1988. Identification and nucleotide sequence of the class E *tet* regulatory elements and operator and inducer binding of the encoded purified Tet repressor. Mol. Gen. Genet. **215:**76–80.
32. **Zwicker, B. M. (Halifax Fisheries Research Laboratory, Halifax, Nova Scotia, Canada).** 1991. Personal communication.