APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1984, p. 975–979
0099-2240/84/110975-05$02.00/0

Vol. 48, No. 5

# Multilocus Electrophoretic Assessment of the Genetic Structure and Diversity of *Yersinia ruckeri*

WILLIAM B. SCHILL,[1]* STEVAN R. PHELPS,[2] AND STEPHEN W. PYLE[3]

*U.S. Fish and Wildlife Service, National Fish Health Research Laboratory, Kearneysville, West Virginia 25430[1]; Washington Cooperative Fishery Research Unit, University of Washington, Seattle, Washington 98195[2]; and Georgia Cooperative Fishery Research Unit, University of Georgia, Athens, Georgia 30602[3]*

Received 21 May 1984/Accepted 28 August 1984

**Multilocus isoenzyme electrophoresis was used to screen 47 field isolates of *Yersinia ruckeri* for electrophoretic variation at 15 enzyme loci. Only four electrophoretic types were observed, thus indicating that the genetic structure of *Y. ruckeri* is clonal. Forty-two isolates were of one electrophoretic type, a reflection of the low amount of genetic diversity extant in this species. Although sorbitol fermentation has been considered to be indicative of a second biotype, no significant gene frequency differences were found between the group of 20 isolates that readily used sorbitol as the sole carbon source and the group of 27 that did not.**

*Yersinia ruckeri* is a significant bacterial pathogen of fish. It is best known as the cause of enteric redmouth disease, a septicemia of salmonids, but it is also been implicated in disease outbreaks in other fish species (19; D. L. Mitchum, Fish Health Section/American Fisheries Society Newsletter 9:2, 1981). In addition, *Y. ruckeri* has been recovered from the lower intestine of a seemingly healthy muskrat, *Ondatra zibethica* (19).

Shipment of carrier salmonids usually has been implicated in the spread of enteric redmouth disease, but Bullock et al. (2) reported cases of enteric redmouth disease in West Virginia and Australia with no obvious link to imported fish. Accordingly, these authors suggested the possibility that *Y. ruckeri* is widely distributed but was generally unrecognized until recently. Nevertheless, drastic measures have been used to check the spread of enteric redmouth disease because an outbreak of the disease can be devastating. Fish shipments have been halted, and both carrier and exposed populations have been destroyed when *Y. ruckeri* has been found, even in outwardly healthy fish (19). Despite these efforts, the geographic range of *Y. ruckeri* now includes North America, Australia, France, Germany, England, and Italy (2, 6, 9, 17; G. Giorgetti, G. Ceschia, and G. Boud, Proc. Eur. Assoc. Fish Pathologists, in press.).

As has been the case with other organisms, several techniques have been brought to bear on *Y. ruckeri* in efforts to establish intraspecies classification criteria to be used in epidemiology and vaccine studies. Identification of virulence determinants has been a high research priority. Although biotypes, serotypes, and plasmid profiles have been defined (2, 4, 15, 19, 20), none of these characterizations has yielded information that is entirely suitable for defining the genetic structure of the species. Biotypic designations are susceptible to slight variation in methodology; plasmid profiles often vary with methodology and culture growth conditions; and serotypic designations are often equivocal or undefined for particular isolates.

Multilocus isoenzyme electrophoresis has been used in hundreds of population genetics studies of plants, animals, and fungi, but until recently it has not been used extensively in the study of the population genetics of bacteria (11, 13, 14, 18, 23, 24). Allelic variants of enzymes (isoenzymes) are detected by shifts in electrophoretic mobility as a consequence of one or more amino acid substitutions and the resultant change in net charge. The proportion of polymorphic loci within a species, the numbers and frequencies of occurrence of alleles present at particular loci, and the presence or absence of temporal or geographic clines can be determined by screening a number of arbitrarily selected enzymes for allelic variation. Estimates of genetic distance derived from isoenzyme electrophoresis have been shown to be correlated with estimates of sequence divergence obtained from DNA hybridization (14). Recently, Ochman and Selander (13) and Kusecek et al. (8) demonstrated that knowledge of the genetic structure of natural bacterial populations may be requisite for identifying virulence determinants and understanding pathogenic processes.

Using starch gel electrophoresis, we examined 15 chromosomal loci for electrophoretic variation in a survey of 47 *Y. ruckeri* field isolates. The isolates were recovered from obviously diseased as well as from apparently healthy fish in the course of disease survey and diagnostic work of the National Fish Health Research Laboratory and other laboratories. Seven salmonid and four nonsalmonid host species were represented; 42 isolates were from the United States, 3 were from Australia, and 2 were from Canada.

## MATERIALS AND METHODS

**Bacterial isolates.** All *Y. ruckeri* isolates were maintained in the National Fish Health Research Laboratory culture collection. Two isolates were originally obtained from the University of Guelph, Ontario, Canada, and have been previously described (19); isolate 11.59 was formerly designated ONT258, and isolate 11.60 was formerly designated ONT288. The designations and origins of the *Y. ruckeri* isolates used in this study are detailed in Table 1.

Biochemical testing of *Y. ruckeri* isolates were conducted by previously described methods (5), except that sucrose fermentation and Tween 80 hydrolysis were determined with SW medium (22), and sorbitol utilization was tested as described below. Unless specified, all tests were conducted at 25°C.

All *Y. ruckeri* isolates examined were motile and had positive reactions for triple sugar iron (*K/A*), Simmon's citrate, ornithine decarboxylase, and Tween 80 hydrolysis

* Corresponding author.

TABLE 1. *Y. ruckeri* isolates tested

| Isolate | Date of isolation | Host species | Place of isolation | Sorbitol biotype | Variable locus designation | | |
|---|---|---|---|---|---|---|---|
| | | | | | MDH | GLPEP-1 | GPI |
| 11.1 | 1963 | *Salmo gairdneri* | Idaho | − | 100 | 100 | 100 |
| 11.4 | 1968 | *Salmo gairdneri* | Utah | − | 100 | 100 | 100 |
| 11.5 | 1968 | *Salmo clarki* | Wyoming | − | 100 | 100 | 100 |
| 11.12 | 1972 | *Salmo gairdneri* | Ohio | − | 100 | 100 | 100 |
| 11.13 | 1972 | *Salmo gairdneri* | Ohio | − | 100 | 100 | 100 |
| 11.14 | 1972 | *Salmo gairdneri* | Ohio | − | 100 | 100 | 100 |
| 11.16 | 1974 | *Salmo gairdneri* | Ohio | − | 100 | 100 | 100 |
| 11.25 | 1976 | *Salmo gairdneri* | Missouri | − | 100 | 100 | 100 |
| 11.26 | 1976 | *Salmo gairdneri* | North Carolina | − | 100 | 100 | 100 |
| 11.28 | 1977 | *Salmo gairdneri* | Pennsylvania | − | 100 | 100 | 100 |
| 11.29 | 1974 | *Oncorhynchus tshawytscha* | Oregon | + | 100 | 100 | 100 |
| 11.30 | 1977 | *Morone americana* | Maine | − | 100 | 100 | 100 |
| 11.31 | 1978 | *Salmo gairdneri* | California | + | 100 | 75 | 100 |
| 11.33 | 1978 | *Salmo gairdneri* | Tennessee | − | 100 | 100 | 100 |
| 11.34 | 1978 | *Salmo gairdneri* | Colorado | − | 100 | 100 | 100 |
| 11.35 | 1978 | *Salmo gairdneri* | Colorado | − | 100 | 100 | 100 |
| 11.36 | 1978 | *Salmo gairdneri* | Colorado | − | 100 | 100 | 100 |
| 11.37 | 1978 | *Salmo gairdneri* | Colorado | − | 100 | 100 | 100 |
| 11.38 | 1978 | *Micropterus salmoides* | New York | − | 140 | 100 | 100 |
| 11.39 | 1978 | *Salmo gairdneri* | North Carolina | − | 100 | 100 | 100 |
| 11.40 | 1978 | *Salmo gairdneri* | North Carolina | − | 100 | 100 | 100 |
| 11.42 | 1952 | *Salvelinus fontinalis* | West Virginia | − | 100 | 100 | 100 |
| 11.43 | 1959 | *Salmo gairdneri* | Australia | − | 100 | 100 | 100 |
| 11.44 | 1959 | *Salmo gairdneri* | Australia | − | 100 | 100 | 100 |
| 11.45 | 1959 | *Salmo gairdneri* | Australia | − | 100 | 100 | 100 |
| 11.46 | 1978 | ? | Alaska | − | 100 | 100 | 100 |
| 11.47 | 1978 | *Salmo gairdneri* | Colorado | + | 100 | 100 | 100 |
| 11.48 | 1979 | *Salmo gairdneri* | Colorado | + | 100 | 100 | 100 |
| 11.49 | 1978 | *Salmo gairdneri* | Colorado | + | 100 | 100 | 100 |
| 11.50 | 1979 | *Salmo gairdneri* | Colorado | + | 100 | 100 | 100 |
| 11.51 | 1979 | *Salmo gairdneri* | Colorado | + | 100 | 100 | 100 |
| 11.52 | 1979 | ? | Alaska | − | 100 | 100 | 100 |
| 11.53 | 1979 | ? | Alaska | − | 100 | 100 | 100 |
| 11.54 | 1979 | *Salmo gairdneri* | Virginia | + | 100 | 100 | 100 |
| 11.55 | 1980 | *Salmo salar* | Massachusetts | − | 100 | 100 | 100 |
| 11.57 | 1980 | *Salmo trutta* | Montana | + | 100 | 100 | 100 |
| 11.58 | 1980 | *Salmo gairdneri* | Montana | + | 100 | 100 | 100 |
| 11.59 | 1981 | *Salmo gairdneri* | Ontario, Canada | + | 140 | 100 | 100 |
| 11.60 | 1981 | *Coregonus artedii* | Ontario, Canada | + | 90 | 100 | 107 |
| 11.61 | 1981 | *Salmo aguabonita* | Wyoming | + | 100 | 100 | 100 |
| 11.62 | 1981 | *Salmo gairdneri* | Idaho | + | 100 | 100 | 100 |
| 11.63 | 1981 | *Salmo gairdneri* | Montana | + | 100 | 100 | 100 |
| 11.64 | 1981 | *Salmo aguabonita* | Montana | + | 100 | 100 | 100 |
| 11.65 | 1981 | *Salmo aguabonita* | Montana | + | 100 | 100 | 100 |
| 11.66 | 1978 | *Salmo gairdneri* | California | + | 100 | 75 | 100 |
| 11.67 | ? | ? | ? | + | 100 | 100 | 100 |
| 11.68 | 1983 | *Carassius auratus* | Maryland | + | 100 | 100 | 100 |

and negative reactions for $H_2S$ production (SIM medium [Difco Laboratories, Detroit, Mich.]), indole (SIM medium), malonate, urease, oxidase, and sucrose fermentation. Sorbitol utilization reactions were variable (Table 1).

**Media and growth conditions.** For electrophoretic analysis, isolates were grown on brain heart infusion agar (Difco) slants for 24 h at 20°C. A minimal salts plus 1% sorbitol medium (MSS) was used to score isolates for sorbitol biotype. MSS contained (per liter of deionized water): 1 g of $NH_4Cl$, 6 g of $Na_2HPO_4$, 3 g of $KH_2PO_4$, 0.5 g of NaCl, 0.246 g of $MgSO_4 \cdot 7H_2O$, 0.0147 g of $CaCl_2 \cdot 2H_2O$, and 10 g of sorbitol.

**Sorbitol biotype assay.** Isolates were grown for 24 h in brain heart infusion broth containing 1% (wt/vol) sorbitol. Cells were collected by centrifugation at 2,500 × *g* for 20 min, washed twice in sterile phosphate-buffered saline, resuspended to original growth volume in sterile phosphate-buffered saline, and used to inoculate MSS medium. Visible growth by 48 h at 20°C was considered to be indicative of a sorbitol-positive biotype.

**Preparation of cell extracts.** Cells were suspended in 1 ml of phosphate-buffered saline and pelleted at 10,000 × *g* for 5 min in a Beckman Microfuge. The supernatants were removed and discarded. The cells were then washed twice by resuspending them in a 1.5 ml of phosphate-buffered saline and pelleting the cells as before. Cell extracts were prepared by adding 0.5 ml of deionized water to each washed cell pellet and freezing at −20°C. No attempt was made to resuspend the cells. We allowed the materials to thaw slightly and then sonicated them for 10 s at a setting of 5 (40 W) using a model W185D Branson sonifier (Branson Sonic Power Co., Plainview, N.Y.) equipped with a microprobe. Intact cells and debris were removed by a 5-min centrifugation at 10,000 × *g* in the Microfuge, and the extracts were

used immediately or stored at −20°C until used. Repeated cycles of freezing and thawing were avoided.

**Isoenzyme electrophoresis.** We conducted horizontal starch gel electrophoresis as described by Utter et al. (21), using 12.5% starch (Connaught Laboratories Limited, Willowdale, Ontario, Canada). Two buffer systems were used. (i) This system is as described by Clayton and Tretiak (3). The gel buffer was 0.002 M citric acid (pH 6.5). The electrode buffer was 0.04 M citric acid (pH 6.5). Both the gel and the electrode buffers were adjusted to pH with *N*-(3-aminopropyl)-morpholine. (ii) This system is as described by Ridgway et al. (16). The gel buffer was 0.03 M Tris–0.005 M citric acid–1% electrode buffer (pH 8.5). The electrode buffer was 0.06 M lithium hydroxide–0.3 M boric acid (pH 8.1).

The enzyme stains used were described by Allendorf et al. (1) and by Harris and Hopkinson (7), except the stain for aspartate aminotransferase. The staining solution for aspartate aminotransferase was prepared as a stock solution containing the following components (per liter): 0.73 g of alpha-ketoglutaric acid, 2.66 g of L-aspartic acid, 10.0 g of polyvinylpyrrolidone (molecular weight, 10,000 to 40,000), 1.0 g of disodium EDTA, and 28.4 g of dibasic sodium phosphate. The stock solution was stored at 4°C. Immediately before use, a working solution was prepared by warming the necessary quantity of stock solution to room temperature and adding fast garnet GBC salt (Sigma F 0875) to a final concentration of 0.5 mg/ml.

The following enzymes were assayed: acid phosphatase (EC 3.1.3.2), adenylate kinase (EC 2.7.4.3), aldolase (EC 4.1.2.13), aspartate aminotransferase (EC 2.6.1.1), creatine kinase (EC 2.7.3.2), diaphorase (EC 1.6.4.3), β-D-galactosidase (EC 3.2.1.23), glucosephosphate isomerase (GPI; EC 5.3.1.9), glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12), alpha-glycerophosphate dehydrogenase (EC 1.1.1.8), isocitrate dehydrogenase (EC 1.1.1.42), lactate dehydrogenase (EC 1.1.1.27), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (EC 1.1.1.40), mannose-6-phosphate isomerase (EC 5.3.1.8), peptidase-glycylleucine (GLPEP; EC 3.4.*.*), peptidase-leucylglycylglycine (EC 3.4.*.*), phosphoglucomutase (EC 2.7.5.1), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), sorbitol dehydrogenase (EC 1.1.1.14), and superoxide dismutase (EC 1.15.1.1).

The nomenclature used to describe the isozyme variation that we observed followed the system proposed by May et al. (10). Loci were numbered consecutively from most cathodal to most anodal. The most common allele was assigned a mobility of 100, and variant alleles were assigned mobilities relative to the common allele. For example, GLPEP-1 (75) refers to an allele at the first GLPEP locus whose protein product migrated 75% as far as the protein product of the common allele at this locus. Negative mobilities were assigned to protein products that migrated toward the cathode, and positive mobilities were assigned to protein products that migrated toward the anode.

## RESULTS

A total of 47 *Y. ruckeri* isolates were examined for activity and mobility of the 21 enzymes listed. Seven of the enzymes were not demonstrable under standard conditions, had only weak activity, or were poorly resolved: acid phosphatase, aldolase, β-D-galactosidase, glyceraldehyde-phosphate dehydrogenase, alpha-glycerophosphate dehydrogenase, lactate dehydrogenase, and sorbitol dehydrogenase. Peptidase-leucylglycylglycine showed the same zones of activity as GLPEP, but at a much lesser activity.

Because sorbitol fermentation has been used as a criterion to define a second biotype of *Y. ruckeri* (P. J. O'Leary, M.S. thesis, Oregon State University, Corvallis, 1977), we scored each isolate as described above for its ability to readily use sorbitol as the only carbon source in the growth medium. Of the 47 isolates tested, we scored 20 as positive for sorbitol use and 27 as negative. There were no significant gene frequency differences between the positive and negative groups at any of the loci examined (the largest Chi-square value was 1.591; 1 df).

With 5 exceptions, the 47 *Y. ruckeri* isolates had identical isozyme patterns at 15 enzyme loci (Table 1 and Fig. 1). Isolates 11.38 and 11.59 demonstrated a MDH allele with a relative mobility of 140. Isolates 11.31 and 11.66 demonstrated a GLPEP-1 allele with a relative mobility of 75. We later determined that isolates 11.31 and 11.66 were in fact only one isolate that had come to the laboratory by divergent routes. No other similar cases were identified. Isolate 11.60 demonstrated a MDH allele with a relative mobility of 90 and a GPI allele with a relative mobility of 107. Our single locus estimates of genetic diversity (12) were 0.124 for MDH, 0.043 for GPI, and 0.043 for GLPEP-1.

## DISCUSSION

In their study of genetic diversity in *Escherichia coli*, Selander and Levin (18) observed only 98 distinctive electrophoretic types among the many thousands theoretically possible for this bacterium. For this reason, they proposed that the genetic structure of natural populations of *E. coli* is for the most part clonal and that genetic recombination of chromosomal genes is limited. A clonal genetic structure

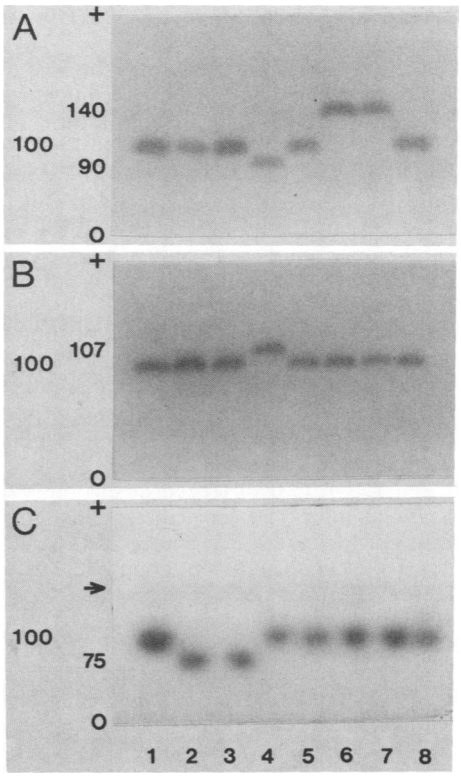


FIG. 1. Isozyme patterns of representative *Y. ruckeri* isolates. Isolates shown are 11.4, 11.31, 11.66, 11.60, 11.29, 11.38, 11.59, and 11.43 (lanes 1 through 8, respectively). (A) MDH; (B) GPI; (C) GLPEP-1. The arrow in (C) indicates the position of GLPEP-2 (faint) activity.

seems to exist in *Y. ruckeri* as well, because we observed only four electrophoretic types that differed at no more than 2 of the 15 loci examined. We therefore designate each electrophoretic type as being representative of a clone.

Clone I consists of isolates demonstrating the common electrophoretic type; because it included 42 of the 46 isolates tested, it was by far the largest and the most widespread geographically. It contained isolates from many locations across the United States and several locations in Australia.

Clone II consists of two isolates from the Great Lakes region. Isolate 11.38 was recovered in 1978 from largemouth bass (*Micropterus salmoides*) in the state of New York, and isolate 11.59 (ONT258) was recovered in 1982 from rainbow trout (*Salmo gairdneri*) at Normandale Hatchery, Ontario, Canada. Although these two isolates were electrophoretically identical for the loci examined, 11.59 fermented sorbitol readily, whereas 11.38 did not. In plasmid profiles of *Y. ruckeri* isolates examined by DeGrandis and Stevenson (4) and Toranzo et al. (20), high-molecular-mass plasmids (20 to 72 megadaltons) were found in predominantly nonsorbitol-fermenting isolates, whereas only low-molecular-mass plasmids ($\leq$ 5.5 megadaltons) were found in sorbitol-fermenting isolates. Stevenson and Daly (19) reported that isolate ONT258 reacted with both serovar I and serovar II antisera. Further studies of this clone may clarify possible correlations between biotype, serotype, plasmid content, and virulence.

Clones III and IV both consisted of single isolates. The sole representative of clone III was isolate 11.31 (11.66), recovered from rainbow trout at Coleman National Fish Hatchery, California, in 1978. Clone IV was represented by isolate 11.60 (ONT288), which was recovered from moribund ciscoes (*Coregonus artedii*) in northern Ontario, Canada.

The purpose of this work was not to assign clinical significance to any group of *Y. ruckeri* isolates tested, but rather to investigate the level and distribution of genetic diversity within *Y. ruckeri*. It is noteworthy, however, that Ochman and Selander (13) and Kusecek et al. (8) have found in their studies of *E. coli* that O1:K1 isolates of the ETe/OMP9 clone were genetically similar to O18:K1 isolates of the ETf/OMP9 clone, but were found in inverse frequencies in cases of urinary tract infections and neonatal meningitis. The genetic similarity of these two clones (as assayed by multilocus isoenzyme electrophoresis) highlighted the lipopolysaccharide (O antigen) difference as a determinant of virulence. Thus, knowledge of the genetic structure of natural bacterial populations may aid in the interpretation of pathogenicity studies and may be essential for an understanding of the disease process.

Selander and Levin (18) observed electrophoretic variation in *E. coli* at all of 20 loci; the average number of electromorphs per locus was 7.3, and the estimated average genetic diversity was 0.4718. In contrast, we observed variation in only 3 of 15 loci in *Y. ruckeri*; the average number of electromorphs per locus was 1.27, and the estimated average genetic diversity was 0.014.

The results of this study indicate that the genetic structure of *Y. ruckeri* is clonal and that one clone is predominant. The average genetic diversity of *Y. ruckeri* is only about 1/30 that of *E. coli* and about 1/4 that of the salmonids that it infects.

## ACKNOWLEDGMENTS

This work was supported in part by the U.S. Department of Energy, Bonneville Power Administration, under Project Agreement 83-304.

For the helpful information they provided, we thank Roselyn Stevenson, University of Guelph, Ontario, Canada; John Schachte, New York State Department of Environmental Conservation, Fish Disease Control Unit, Rome, N.Y.; and Paul Janeke, Fish Disease Control Center, Fort Morgan, Colo. We also thank G. L. Bullock for the advice that helped initiate this study and for his continued support throughout the work.

## LITERATURE CITED

1. **Allendorf, F. W., N. Mitchell, N. Ryman, and G. Ståhl.** 1977. Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. Hereditas **86**:179–190.
2. **Bullock, G. L., H. M. Stuckey, and E. B. Shotts, Jr.** 1978. Enteric redmouth bacterium: comparison of isolates from different geographic areas. J. Fish Dis. **1**:351–356.
3. **Clayton, J. W., and D. N. Tretiak.** 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. **29**:1169–1172.
4. **DeGrandis, S. A., and R. M. W. Stevenson.** 1982. Variations in plasmid profiles and growth characteristics of *Yersinia ruckeri* strains. FEMS Microbiol. Lett. **15**:199–202.
5. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis, Minn.
6. **Fuhrmann, H., K. H. Böhm, and H.-J. Schlotfeldt.** 1983. An outbreak of enteric redmouth disease in West Germany. J. Fish Dis. **6**:309–312.
7. **Harris, H., and D. A. Hopkinson.** 1976. Handbook of enzyme electrophoresis in human genetics, 1st ed. North-Holland Publishing Co., Amsterdam.
8. **Kusecek, B., H. Wloch, A. Mercer, V. Vaisänen, G. Pluschke, T. Korhonen, and M. Achtman.** 1984. Lipopolysaccharide, capsule, and fimbriae as virulence factors among O1, O7, O16, O18, or O75 and K1, K5, or K100 *Escherichia coli*. Infect. Immun. **43**:368–379.
9. **Lesel, R., M. Lesel, F. Gavini, and A. Vuillaume.** 1983. Outbreak of enteric redmouth disease in rainbow trout, *Salmo gairdneri* Richardson, in France. J. Fish Dis. **6**:385–388.
10. **May, B., J. E. Wright, and M. Stoneking.** 1979. Joint segregation of biochemical loci in Salmonidae: results from experiments with *Salvelinus* and review of the literature on other species. J. Fish. Res. Board Can. **36**:1114–1128.
11. **Milkman, R.** 1973. Electrophoretic variation in *Escherichia coli* from natural sources. Science **182**:1024–1026.
12. **Nei, M.** 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**:583–590.
13. **Ochman, H., and R. K. Selander.** 1984. Evidence for clonal population structure in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **81**:198–201.
14. **Ochman, H., T. S. Whittam, D. A. Caugant, and R. K. Selander.** 1983. Enzyme polymorphism and genetic population structure in *Escherichia coli* and *Shigella*. J. Gen. Microbiol. **129**:2715–2726.
15. **O'Leary, P. J., J. S. Rohovec, and J. L. Fryer.** 1979. A further characterization of *Yersinia ruckeri* (enteric redmouth bacterium). Fish Pathol. **14**:71–78.
16. **Ridgway, G. J., S. W. Sherburne, and R. D. Lewis.** 1970. Polymorphisms in the esterases of Atlantic herring. Trans. Am. Fish. Soc. **99**:147–151.
17. **Roberts, M. S.** 1983. A report of an epizootic in hatchery reared rainbow trout, *Salmo gairdneri* Richardson, at an English trout farm, caused by *Yersinia ruckeri*. J. Fish Dis. **6**:551–552.
18. **Selander, R. K., and B. R. Levin.** 1980. Genetic diversity and structure in *Escherichia coli* populations. Science **210**:545–547.
19. **Stevenson, R. M. W., and J. G. Daly.** 1982. Biochemical and serological characteristics of Ontario isolates of *Yersinia ruckeri*. Can. J. Fish. Aquat. Sci. **39**:870–876.
20. **Toranzo, A. E., J. L. Barja, R. R. Colwell, and F. M. Hetrick.** 1983. Characterization of plasmids in bacterial fish pathogens. Infect. Immun. **39**:184–192.
21. **Utter, F. M., H. O. Hodgins, and F. W. Allendorf.** 1974. Biochemical genetic studies of fishes: potentials and limitations,

p. 213–238. *In* D. C. Malins and J. R. Sargent (ed.), Biochemical and biophysical perspectives in marine biology. Academic Press, Inc., New York.

22. **Waltman, W. D., and E. B. Shotts.** 1984. A medium for the isolation and differentiation of Yersinia ruckeri. Can. J. Fish. Aquat. Sci. **44**:804–806.
23. **Whittam, T. S., H. Ochman, and R. K. Selander.** 1983. Multilocus structure in natural populations of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **80**:1751–1755.
24. **Whittam, T. S., H. Ochman, and R. K. Selander.** 1983. Geographic components of linkage disequilibrium in natural populations of *Escherichia coli*. Mol. Biol. Evol. **1**:67–83.