APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1996, p. 1167–1170 Vol. 62, No. 4
0099-2240/96/$04.00+0

Identification of *Aeromonas hydrophila* Hybridization Group 1 by PCR Assays

ALBERTO CASCÓN, JUAN ANGUITA, CARMEN HERNANZ, MARÍA SÁNCHEZ, MÁXIMO FERNÁNDEZ, AND GERMÁN NAHARRO*

Departamento de Patología Animal (Sanidad Animal), Unidad de Microbiología Inmunología, Universidad de León, 24007 León, Spain

Received 22 September 1995/Accepted 10 January 1996

Synthetic oligonucleotide primers of 24 and 23 bases were used in a PCR assay to amplify a sequence of the *lip* gene, which encodes a thermostable extracellular lipase of *Aeromonas hydrophila*. A DNA fragment of approximately 760 bp was amplified from both sources, i.e., lysed *A. hydrophila* cells and isolated DNA. The amplified sequence was detected in ethidium bromide-stained agarose gels or by Southern blot analysis with an internal *Hin*dIII-*Bam*HI 356-bp fragment as a hybridization probe. With *A. hydrophila* cells, the sensitivity of the PCR assay was <10 CFU, and with the isolated target, the lower detection limit was 0.89 pg of DNA. Primer specificity for *A. hydrophila* was determined by the PCR assay with cells of 50 strains of bacteria, including most of the 14 currently recognized DNA hybridization groups of *Aeromonas* spp. as well as other human and environmental *Aeromonas* isolates. Detection of *A. hydrophila* by PCR amplification of DNA has great potential for rapid identification of this bacterium because it has proved to be highly specific.

Aeromonas hydrophila and related species are gram-negative, facultatively anaerobic freshwater bacteria which are related to disease in humans and are also pathogenic for aquatic and terrestrial animals (1, 11, 17). Most human and environmental isolates of *A. hydrophila* and related aeromonads secrete many extracellular products, some of which, such as aerolysin, enterotoxins, proteases, and acetylcholinesterase, have been characterized and considered as virulence factors in pathogenesis (4, 14, 15, 17, 22, 23). Furthermore, *A. hydrophila* releases an extracellular glycerophospholipid-cholesterol acyltransferase, which has been characterized by others (8, 24, 26). Glycerophospholipid-cholesterol acyltransferase is analogous to the mammalian plasma enzyme lecithin-cholesterol acyltransferase (12) and, like other lipases found in the microbial world, is a member of the lipase superfamily. In addition, we have purified and characterized another extracellular lipase, and its gene, the *lip* gene, has been cloned and sequenced (2). Microbial lipases have received increasing attention because of their potential biotechnological applications as stereospecific catalysts, flavor-modifying enzymes, or detergent additives (6), but their role in bacterial metabolism is not fully understood. Lipases may be important extracellular enzymes for bacterial nutrition (7), and they may also be virulence factors affecting several immune system functions through free fatty acids generated by lipolytic activity (3, 5).

Since *A. hydrophila* was first recognized as a significant opportunistic pathogen for humans, many efforts were dedicated to find methods for a correct identification (16, 18) and also for classification of species belonging to this genus. For many years, the taxonomy of *Aeromonas* spp. was confusing, and after significant revision, it now appears reasonably ordered (13). Popoff and coworkers (20), studying 55 *Aeromonas* strains, placed them into DNA hybridization groups (HGs). Since then, the taxonomy of the genus has depended on a complex mixture of phenotypic and genotypic data. Biochemically distinct species are referred to as phenospecies, whereas distinct DNA HGs are called genospecies or genomic species. *Bergey's Manual of Systematic Bacteriology* (19), on the basis of phenotypic characteristics, recognizes the following four groups or complexes in the genus *Aeromonas*: *A. hydrophila* (HGs 1, 2, and 3), *Aeromonas caviae* (HGs 4, 5A, 5B, and 6), *Aeromonas sobria* (HGs 7, 8, and 9), and *Aeromonas salmonicida* (HG 3). These groups are treated as if they were members of one (pheno)species, although each consists of one or more HGs. In comparison, the following recently described taxa seem to be genotypically more homogeneous: *Aeromonas veronii* biogroup veronii (HG 10), *Aeromonas schubertii* (HG 12), and *Aeromonas trota* (HG 13) (9). Actually, the genus *Aeromonas* contains at least 11 phenospecies and at least 14 HGs (10).

Since more and more *A. hydrophila* strains are being found in different samples (water, fecal and clinical specimens, and food), the development of sensitive and specific methods for rapid detection of *A. hydrophila* is needed. Amplification of specific DNA by PCR provides a highly sensitive and specific tool for the detection of microorganisms from different sources. In this report, we describe the development of a specific, rapid, and sensitive nucleic acid-based procedure (PCR) to detect the lipase H3 gene in *A. hydrophila* cells without nucleic acid extraction. The utility of the PCR is further enhanced by the hybridization assay for the PCR product.

MATERIALS AND METHODS

Strains and culture conditions. The *Aeromonas* strains used in this study are detailed in Table 1. All reference strains were obtained from the Spanish Type Culture Collection (CECT), and another designation is given when known. Environmental strains have been isolated in our laboratory and identified as described previously (19). Two strains of *A. hydrophila*, 67-P-24 and 1.54, were fish isolates (25). Human isolates were provided by the Hospital Princesa Sofía, León, Spain, and identified as before. Strains were grown on Luria broth (LB) or Luria agar. To detect extracellular lipolytic activity, Luria agar was supplemented with 0.5% tributyrin emulsified by sonication in the presence of 0.2% Triton X-100; a clear halo around colonies indicated lipolytic activity (2). Cells were incubated at 28°C except for strains of *A. salmonicida*, which were incubated at 20°C. *Escherichia coli* C600-1, used to propagate plasmid pLA2 (2), was grown on LB medium and incubated at 37°C. *E. coli* C600-1 and *E. coli* AB2829 were included in the PCR assay as negative controls. *Streptococcus pyogenes* CECT 190 was grown on nutrient agar and incubated at 37°C, and *Pseudomonas putida* CECT 324 and *Serratia marcescens* CECT 159 were grown on LB and incubated at 26°C.

* Corresponding author. Phone: 34 987 291294. Fax: 34 987 291194.

TABLE 1. Summary of microorganisms used in this study and their lipolytic activities and PCR products

Aeromonas strains	HG	Lipolytic activity[a]	PCR product (760 bp)
Reference strains			
A. hydrophila CECT 839 (ATCC 7966)	1	+	+
A. hydrophila CECT 398 (NCIB 9233)	2	+	−
A. hydrophila CECT 4223 (ATCC 14486)	2	+	−
A. euchrenophila CECT 4224 (ATCC 23309)	6	+	−
A. jandaei CECT 4228 (ATCC 49568)	9	+	−
A. salmonicida CECT 895 (ATCC 33659)	3	+	−
A. salmonicida CECT 894 (ATCC 33658)	3	+	−
A. salmonicida CECT 896 (ATCC 27013)	3	+	−
A. sobria CECT 837 (NCIB 12065)	7	+	−
A. trota CECT 4255 (ATCC 49657)	13	+	−
A. enteropelogenes CECT 4487 (ATCC 49803)	ND[b]	+	−
A. shuberti CECT 4241 (ATCC 43700)	12	+	−
A. ichthiosmia CECT 4486 (ATCC 49904)	ND	+	−
A. media CECT 4232 (ATCC 33907)	5b	+	−
A. allosaccharophila CECT 4199	ND	+	−
A. caviae CECT 838 (ATCC 15468)	4	+	−
Environmental strains[c]			
A. hydrophila D6	1	+	+
A. hydrophila D9	1	+	+
A. hydrophila D11	1	+	+
A. hydrophila D13	1	+	+
A. hydrophila A7	1	+	+
A. hydrophila F9	1	+	+
A. hydrophila S04/1	1	+	+
A. hydrophila 67-p-24	1	+	+
A. hydrophila B01.3	1	+	+
A. hydrophila B01.6	1	+	+
A. hydrophila B01.9	1	+	+
A. hydrophila B01.27	1	+	+
A. hydrophila B01.28	1	+	+
A. hydrophila 1.54	1	+	+
A. hydrophila S02/2	1	+	+
A. caviae B01.5	4	+	−
A. caviae B01.31	4	+	−
A. caviae B01.32	4	+	−
A. caviae D5	4	+	−
A. caviae D8	4	+	−
A. caviae D10	4	+	−
A. caviae B01.10	4	+	−
A. caviae B01.11	4	+	−
A. caviae B01.23	4	+	−
A. caviae B02.8	4	+	−
A. caviae H01	4	+	−
A. caviae H02	4	+	−
A. caviae H03	4	+	−
A. sobria HS1	7	+	−
A. sobria HS2	7	+	−
A. sobria B01.1	7	+	−
Human isolates[d]			
A. hydrophila H1	1	+	+
A. hydrophila H2	1	+	+
A. hydrophila H3	1	+	+

[a] Lipolytic activity was determined as described previously (14).
[b] ND, not determined.
[c] Freshwater isolates; identified as described previously (24).
[d] Isolated from a blood culture from hospitalized patients.

DNA isolation and manipulation. Chromosomal DNA from *Aeromonas* strains was obtained from an overnight culture grown on LB medium as reported earlier (21). Plasmid DNA was extracted by an alkaline lysis procedure and purified by two rounds of ultracentrifugation through CsCl-ethidium bromide gradients followed by extensive dialysis (for 6 h, and with buffer changed every hour) against Tris-EDTA buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA). The internal *Hin*dIII-*Bam*HI 356-bp fragment from plasmid pLA2 (2), used as a probe in hybridization studies, was purified from an agarose gel by use of a Geneclean II kit (Bio 101, Inc., La Jolla, Calif.) as described in the instructions of the manufacturer.

Oligonucleotide probes. Primers were selected on the basis of the nucleotide sequence of the 1,952-bp lipase gene derived from *A. hydrophila* H3 and published elsewhere (2). Primers were synthesized by British Bio-Technological Products (Avingdon, England), and the sequences of the two primers of 24 and 23 bases, respectively, were 5′-AACCTGGTTCCGCTCAAGCCGTTG-3′ (primer 1) and 5′-TTGCTCGCCTCGGCCCAGCAGCT-3′ (primer 2). Primer 1 was between nucleotides +442 and +467, and primer 2 was between nucleotides +1181 and +1205.

Preparation of PCR samples and PCR amplification. Samples to be analyzed were cultures of bacteria and bacterium-extracted nucleic acids. Bacteria were

diluted in sterile saline prior to lysis by 10 min of boiling in a water bath. For determining the sensitivity of the PCR, 10-fold serial dilutions (10^6 to 0 bacteria) were tested. One hundred microliters of each boiled dilution was centrifuged (12,000 × *g* for 15 min) to remove cellular debris, and 5 μl of supernatant was used for PCR amplification without further processing. Viable cells were counted as CFU by triplicate plating of samples on Luria agar and counting colonies after incubation at 28°C for 24 h. When nucleic acids were used, the sensitivity of the PCR was determined by amplifying 5 μl of 10-fold serial dilutions (1 ng to 0.1 pg). PCR amplification was performed with a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) by use of a PCR kit (Boehringer GmbH, Mannheim, Germany) and as described in the instructions of the manufacturer with some modifications. The reaction mixture consisted of 5 μl of DNA-containing sample, 1.25 U of *Taq* DNA polymerase, 5 μl of 10× PCR amplification buffer (100 mM Tris-HCl, 20 mM $MgCl_2$, 500 mM KCl [pH 8.3]), 1 μM each primer, 0.4 mM deoxynucleoside triphosphate, and double-distilled water up to a final volume of 50 μl. To prevent evaporation, 50 μl of mineral oil was added to the mixtures. A total of 40 PCR cycles were run under the following conditions: DNA denaturation at 94°C for 1 min, primer annealing at 62°C for 1 min, and DNA extension at 72°C for 1.5 min. After the final cycle, reactions were terminated by keeping them at 72°C for 5 min.

Southern blot. PCR products were analyzed by agarose gel electrophoresis in a horizontal 1.2% agarose gel and with ethidium bromide incorporated for DNA staining. The DNA was blotted onto a nylon membrane and hybridized with DNA labeled by random priming with digoxigenin-dUTP. Hybrids were detected by enzyme immunoassay as described in the manufacturer's instructions (Boehringer). Stringent conditions were used for hybridization, i.e., 50% formamide and a temperature of 42°C.

RESULTS AND DISCUSSION

Development of the PCR assay and specificity of the PCR. Although the PCR amplification procedure was performed according to the manufacturer's suggestions, minor modifications were introduced. The optimal concentration of $MgCl_2$ was 2 mM in the reaction mixture; no amplification product was obtained when the concentration was 1.5 mM, and nonspecific amplification was obtained when the concentration of $MgCl_2$ was higher than 2 mM. The primer pair was designed on the basis of the published nucleotide sequence of the *lip* gene (2) and used to amplify target sequences in genomic DNA from 50 strains of *Aeromonas* spp. (Table 1). An expected product with a length of 760 bp was obtained in the PCR when *A. hydrophila* H3 cells, the source of the cloned *lip* gene, were used as the source of target DNA (Table 1). *A. hydrophila* H3 presumably belongs to hybridization group 1 (HG 1) on the basis of a set of 11 key tests carried out and currently used to identify all recognized *Aeromonas* genospecies (13). All environmental strains of *A. hydrophila* isolated in our laboratory and those from human sources were identified as DNA HG 1 members; a PCR amplification product of the expected length (760 bp) was obtained (Table 1). *A. hydrophila* ATCC 7966, the type strain and definition strain for DNA HG 1 (Table 1), was also positive in the PCR amplification assay. Two other reference strains of *A. hydrophila* (CECT 398 and CECT 4223) were negative in the PCR amplification assay (Table 1). These two strains are included in the DNA HG2 after identification. No amplification product was obtained when bacterial cells or isolated nucleic acids from reference strains, representing most of the HGs, or *A. caviae* and *A. sobria*, isolated in our laboratory, were used as template DNA (Table 1). These results suggest that the pair of primers used in this study is specific for PCR amplification of a 760-bp fragment from an *A. hydrophila* H3 cloned *lip* gene and appears to be specific for *A. hydrophila* strains included in DNA HG 1. We did not obtain nonspecific amplification products. As a routine negative control, *E. coli* cells or isolated nucleic acid was used.

Southern blot hybridization of PCR amplification products with an internal 356-bp *Hin*dIII-*Bam*HI probe was performed as described in Materials and Methods. All amplification products hybridized with the internal probe (data not shown), demonstrating that these products originated from the previously cloned *lip* gene.

A PCR protocol has been designed to detect the aerolysin gene in *A. hydrophila*-extracted nucleic acid (18); however, strains of *Streptococcus pyogenes* producing erythrogenic toxin and streptolysin O were also typified by amplification of a 200-bp fragment that could be differentiated from the 209-bp *aer* gene amplification product by 4% agarose gel electrophoresis or *Nci*I endonuclease restriction. To check whether our pair of primers could target DNA from *Streptococcus pyogenes*, we used strain CECT 190 of this microorganism as well as other lipase producers such as *Serratia marcescens* CECT 159 and *P. putida* CECT 324 in a PCR assay. PCR products were not obtained in any case (data not shown). Up to this point, these results demonstrated that the primer pair used in our study and targeting the *lip* gene could not target any DNA other than that of *A. hydrophila* included in the DNA HG 1 and could be considered an excellent method of identifying strains of *A. hydrophila* included in HG 1.

Lipolytic activity and PCR product. Aeromonads are known to express many extracellular enzymatic activities. All strains of *Aeromonas* spp. analyzed to date in our laboratory secrete lipolytic activity (Table 1) into the culture medium as determined in LB agar supplemented with 0.5% tributyrin. By this procedure, it was not possible to differentiate among the lipolytic activities secreted by *Aeromonas* spp., as was the case for aerolysin and other beta-hemolytic activities secreted by *Aeromonas* spp. as determined with an agar medium supplemented with erythrocytes (personal observation). The fact that all strains of *Aeromonas* spp. showed lipolytic activity demonstrated that more than one lipolytic activity was being secreted into the medium. Although two lipases, belonging to the lipase superfamily, have been described to date as being present in culture supernatant of *A. hydrophila* strains (2, 26), we also detected esterase activity when purified β-lactamase from *A. hydrophila* was assayed with *p*-nitrophenyl derivatives as substrates (unpublished results). The presence of more than one lipolytic activity might be considered the reason why all strains of *Aeromonas* spp. showed this activity and only lipase H3 producers were positive in the PCR assay. Strains of *Aeromonas* spp. that were negative in the PCR amplification assay and positive for lipolytic activity lack production of lipase H3, which might be masked by other lipolytic activities. That is the reason why the isolation and characterization of species-specific genes must be a useful tool for identifying all recognized *Aeromonas* genospecies.

Sensitivity of the PCR assay. The lower limit of detection of *A. hydrophila* bacterial cells or isolated DNA by PCR was examined for strain H3. A suspension of bacterial cells was diluted and processed as detailed in Materials and Methods. Amplification which resulted in detectable levels of PCR product was achieved when a minimum of 8 CFU of *A. hydrophila* were lysed (Fig. 1A), on the basis of an average of five repeated testings of viable cells and PCR assays. Similar detection limits were observed when other strains of *A. hydrophila* were used. A PCR amplification product could not be obtained when a sample with more than 10^6 CFU was used in the assay, probably because of accumulation of soluble cell products inhibitory to PCR. Isolated DNA from *A. hydrophila* H3 was serially diluted in saline and used as a template. The minimum amount of purified DNA in the reaction mixture needed to obtain a detectable PCR product was 0.89 pg (Fig. 1B).

Our PCR assay also detected *A. hydrophila* in natural samples, such as river water, without culturing. The limit of detection was related to environmental temperature, season of the year, and water pollution.

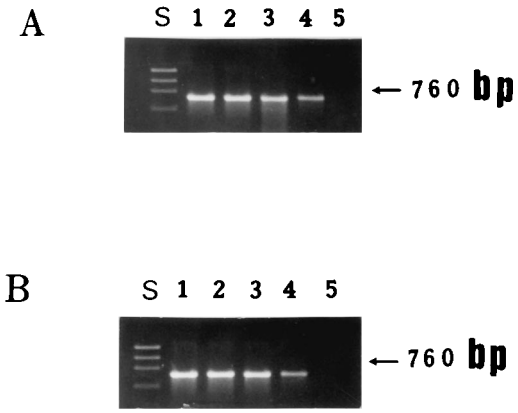

FIG. 1. (A) Agarose gel analysis of PCR products of boiled 10-fold serial dilutions of *A. hydrophila* H3 grown in LB broth. Lanes: 1 to 5, 10^4 to 1 CFU, respectively, of *A. hydrophila* H3. (B) Agarose gel analysis of PCR products of 10-fold serial dilutions of extracted nucleic acid from *A. hydrophila* H3. Lanes: 1 to 5, 1 ng to 0.1 pg, respectively, of extracted nucleic acid. Standards from *Hae*III-digested ϕX174 (top to bottom, 1,353, 1,078, 872, and 603 bp) are shown in lanes S.

ACKNOWLEDGMENTS

We thank the Exma Diputación Provincial de León for financial support. Alberto Cascón is the holder of a fellowship from the Exma Diputación Provincial de León.

REFERENCES

1. **Altwegg, M., and H. K. Geiss.** 1989. *Aeromonas* as a human pathogen. Crit. Rev. Microbiol. **16:**253–286.
2. **Anguita, J., L. B. Rodríguez Aparicio, and G. Naharro.** 1993. Purification, gene cloning, amino acid sequence analysis, and expression of an extracellular lipase from an *Aeromonas hydrophila* human isolate. Appl. Environ. Microbiol. **59:**2411–2417.
3. **Buttke, T. M., and M. A. Cuchens.** 1984. Inhibition of lymphocyte by free fatty acids. II. Toxicity of stearic acid towards phytohaemagglutinin-activated T cells. Immunology **53:**507–517.
4. **Chakraborty, T., B. Huhle, H. Hof, H. Bergbauer, and W. Goebel.** 1987. Marker exchange mutagenesis of the aerolysin determinant in *Aeromonas hydrophila* demonstrates the role of aerolysin in *A. hydrophila*-associated systemic infections. Infect. Immun. **55:**2274–2280.
5. **Eftimiadi, C., E. Buzzi, M. Tonetti, P. Buffa, M. T. J. van Steenbergen, J. Graaff, and G. A. Botta.** 1987. Short-chain fatty acids produced by anaerobic bacteria alter the physiological responses of human neutrophils to chemotactic peptides. J. Infect. Dis. **14:**43–53.
6. **Harwood, J.** 1989. The versatility of lipases for industrial uses. Trends Biochem. Sci. **14:**125–126.
7. **Hedström, S. Å., and P. Nilsson.** 1975. Lipolytic activity of *Staphylococcus aureus* strains from cases of human chronic osteomyelitis and other infections. Acta Pathol. Microbiol. Scand. Sect. B **83:**285–292.
8. **Hilton, S., and J. T. Buckley.** 1991. Studies on the reaction mechanism of a microbial lipase/acyltransferase using chemical modification and site-directed mutagenesis. J. Biol. Chem. **34:**997–1000.
9. **Huys, G., M. Vancanneyt, R. Coopman, P. Janssen, E. Falsen, M. Altwegg, and K. Kersters.** 1994. Cellular fatty acid composition as a chemotaxonomic marker for the differentiation of phenospecies and hybridization groups in the genus *Aeromonas*. Int. J. Syst. Bacteriol. **44:**651–658.
10. **Janda, J. M.** 1991. Recent advances in the study of the taxonomy, pathogenicity, and infectious syndromes associated with the genus *Aeromonas*. Clin. Microbiol. Rev. **4:**397–410.
11. **Janda, J. M., and P. S. Duffey.** 1988. Mesophilic aeromonads in human disease: current taxonomy, laboratory identification, and infectious disease spectrum. Rev. Infect. Dis. **10:**980–997.
12. **Jauhiainen, M., and P. J. Dolphin.** 1986. Human plasma lecithin-cholesterol acyltransferase. J. Biol. Chem. **261:**7032–7043.
13. **Joseph, S. W., and A. Carnahan.** 1994. The isolation, identification, and systematics of the motile *Aeromonas* species. Annu. Rev. Fish Dis. **4:**315–343.
14. **Leung, K. Y., and R. M. W. Stevenson.** 1988. Tn*5*-induced protease-deficient strains of *Aeromonas hydrophila* with reduced virulence for fish. Infect. Immun. **56:**2639–2644.
15. **Nieto, T. P., Y. Santos, L. A. Rodríguez, and A. E. Ellis.** 1991. An extracellular acetylcholinesterase produced by *Aeromonas hydrophila* is a major lethal toxin for fish. Microb. Pathog. **11:**101–110.
16. **O'Brien, D., J. Mooney, D. Ryan, E. Powell, M. Hiney, P. R. Smith, and R. Powell.** 1994. Detection of *Aeromonas salmonicida*, causal agent of furunculosis in salmonid fish, from the tank effluent of hatchery-reared Atlantic salmon smolts. Appl. Environ. Microbiol. **60:**3874–3877.
17. **Paniagua, C., O. Rivero, J. Anguita, and G. Naharro.** 1990. Pathogenicity factors and virulence for rainbow trout (*Salmo gairdneri*) of motile *Aeromonas* spp. isolated from a river. J. Clin. Microbiol. **28:**350–355.
18. **Pollard, D. R., W. M. Johnson, H. Lior, S. D. Tyler, and K. R. Rozee.** 1990. Detection of the aerolysin gene in *Aeromonas hydrophila* by the polymerase chain reaction. J. Clin. Microbiol. **28:**2477–2481.
19. **Popoff, M.** 1984. Genus III. *Aeromonas* Kluyver and van Niel 1936, p. 545–548. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
20. **Popoff, M. Y., C. Coynault, M. Kiredjian, and M. Lemelin.** 1981. Polynucleotide sequence relatedness among motile *Aeromonas* species. Curr. Microbiol. **5:**109–114.
21. **Priefer, U., R. Simon, and A. Puhler.** 1984. Cloning with cosmid, p. 190–210. *In* A. Puhler and K. N. Timmis (ed.), Advanced molecular genetics. Springer-Verlag KG, Berlin.
22. **Rivero, O., J. Anguita, D. Mateos, C. Paniagua, and G. Naharro.** 1991. Cloning and characterization of an extracellular temperature-labile serine protease gene from *Aeromonas hydrophila*. FEMS Microbiol. Lett. **81:**1–8.
23. **Rivero, O., J. Anguita, C. Paniagua, and G. Naharro.** 1990. Molecular cloning and characterization of an extracellular protease gene from *Aeromonas hydrophila*. J. Bacteriol. **172:**3905–3908.
24. **Robertson, D. L., S. Hilton, and J. T. Buckley.** 1992. Stereochemical and positional specificity of the lipase/acyltransferase produced by *Aeromonas hydrophila*. Biochemistry **31:**4974–4980.
25. **Santos, Y., A. E. Toranzo, J. L. Barja, T. P. Nieto, and T. G. Villa.** 1988. Virulence properties and enterotoxin production of *Aeromonas* strains isolated from fish. Infect. Immun. **56:**3285–3293.
26. **Thornton, J., S. P. Howard, and J. T. Buckley.** 1988. Molecular cloning of a phospholipid-cholesterol acyltransferase from *Aeromonas hydrophila*. Sequence homologies with lecithin-cholesterol acyltransferase and other lipases. Biochim. Biophys. Acta **959:**153–159.