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Detection of *Salmonella* spp. in Oysters by PCR

ASIM K. BEJ,[1] MEENA H. MAHBUBANI,[2] MARTIN J. BOYCE,[3] AND RONALD M. ATLAS[3]*

Department of Biology,[1] and School of Dentistry,[2] University of Alabama at Birmingham, Birmingham, Alabama 35294 and Department of Biology, University of Louisville, Louisville, Kentucky 40292[3]

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PCR DNA amplification of a region of the *himA* gene of *Salmonella typhimurium* specifically detected *Salmonella* spp. In oysters, 1 to 10 cells of *Salmonella* spp. were rapidly detected by the PCR following a preenrichment step to increase sensitivity and to ensure that detection was based on the presence of viable *Salmonella* spp.

Salmonella species are often the etiologic agents of gastroenteritis associated with the consumption of contaminated shellfish, particularly raw oysters (2, 13). The recommended method of monitoring shellfish for the presence of microbial pathogens requires culturing followed by a series of presumptive and confirmatory tests that take 5 to 7 days to complete (1, 11, 27). A direct hybridization method has been developed by using a segment of the genomic DNA of *Salmonella typhimurium* as a probe for the detection of *Salmonella* spp. in food (15, 16, 20, 24, 31). At least 10^3 to 10^4 target cells must be present to yield positive hybridization results. Since the infective dose for salmonellosis varies between 10^1 and 10^5 cells (9, 10, 12, 17, 21), a more sensitive molecular method for monitoring *Salmonella* spp. in shellfish would be useful. DNA amplification by the PCR has been used to identify microbial pathogens from clinical, environmental, and food samples (3, 6, 7, 28), including *Campylobacter* spp. in chicken meat (19), *Aeromonas salmonicida* in fish (22), enteropathogenic *Escherichia coli* in ground beef (18), *Shigella flexneri* in lettuce (25), *Vibrio vulnificus* in oysters (23), and enteric viruses in oysters (4). In this report, we describe the development of a PCR-based method that can be used for sensitive and rapid detection of *Salmonella* spp. in oysters.

PCR amplification and detection. Oligonucleotide primers were designed for PCR amplification of *Salmonella* spp. DNAs on the basis of the previously reported nucleotide sequences of the *himA* gene (EMBL and GenBank accession numbers X16739 and M27279) of *S. typhimurium* (26) and other nontarget enteric bacteria. The target gene, *himA*, codes for a DNA binding protein that occurs in many enteric bacteria, including *E. coli* and *S. typhimurium* (26). The nucleotide sequences of the *himA* genes in *S. typhimurium* and *E. coli* have significant homologies, but are not completely homologous. Since Ampli*taq* DNA polymerase does not extend primers across its template DNA if there is even a single nucleotide mismatch at the 3′-OH ends (8, 30), oligonucleotide primers were designed to provide sufficient nucleotide mismatches with the *himA* gene in *E. coli* so that *Salmonella* spp. could be detected specifically; the oligonucleotide primers were designed at regions where the *S. typhimurium* nucleotide sequences do not match the 3′ ends of the *himA* gene in *E. coli*. The primers were synthesized by Roche Diagnostics, Alameda, Calif. Primer annealing temperatures were calculated by using the Oligo version 4 computer program (National Biosciences, Plymouth, Minn.) (29). One primer, SHIMA-L, has 21 nucleotides (5′-CGT-G CTCTGGAAAACGGTGAG-3′); it has a calculated annealing temperature of 61°C and is located between 106 and 126 bp on the *himA* gene of *S. typhimurium*. The other primer, SHIMA-R, has 24 nucleotides (5′-CGTGCTGTAATAGG AATATCTTCA-3′); it has a calculated annealing temperature of 61°C and is located between 204 and 227 bp on the *himA* gene of *S. typhimurium*. A 25-nucleotide probe, SHIMA-P (5′-GGTAACTTCGGTCTGCGTGATAAAA-3′), was selected from the previously reported sequence of the *himA* gene of *S. typhimurium* (26). Oligonucleotide probe SHIMA-P was selected internal to the amplified DNA without overlapping the primer regions; it is located between 148 and 172 bp on the *himA* gene, internal to the two primers. This probe was synthesized by Roche Diagnostics.

PCR amplification was performed with a model 480 or 960 DNA thermal cycler (Perkin Elmer, Norwalk, Conn.) by using 1× PCR reaction buffer (1× PCR reaction buffer consists of 50 mM Tris-HCl [pH 8.9], 50 mM KCl, and 2.5 mM $MgCl_2$) containing each of the deoxynucleoside triphosphates at a concentration of 200 μM, each of the primers at a concentration of 0.5 μM, 100 ng of target DNA or cells from serially diluted samples of *S. typhimurium*, and 2.5 U of Ampli*taq* DNA polymerase (Perkin Elmer). A total of 25 to 35 cycles of amplification of the target DNA were performed, with initial denaturation at 94°C for 3 min and postamplification extension at 72°C for 3 min. The parameters for each amplification cycle were as follows: template DNA denaturation at 94°C for 0.5 min; primer annealing at 65°C for 0.5 min; and primer extension at 72°C for 0.5 min. In all amplification reactions, the hot start approach was used (14).

PCR-amplified DNAs were analyzed by gel electrophoresis and DNA-DNA hybridization by using radiolabelled gene probes. Hybridization was used both to ensure correct detection and to enhance the sensitivity of detection. For gel electrophoresis, the amplified DNA (usually 10 μl from a 100-μl sample) was separated in 3% Nusieve 3:1 (3 parts of Nusieve and 1 part of SeaKem agarose) on a horizontal agarose gel for 30 to 45 min at 9 V/cm by using 1× Tris-acetate-EDTA (TAE) buffer (1× TAE buffer consists of 0.04 M Tris-Cl, 0.02 M sodium acetate, and 0.001 M EDTA; pH adjusted to 7.4 with glacial acetic acid) (5). The DNA

* Corresponding author. Mailing address: Department of Biology, University of Louisville, 139 Life Science Bldg., Belknap Campus, Louisville, KY 40292. Phone: (502) 588-8962. Fax: (502) 588-0725. Electronic mail address: RMATLA01@ULKYVM.

bands in the gel were stained with a 0.0002% ethidium bromide solution and visualized with a Photo/Prep I UV transilluminator (Fotodyne, Inc., New Berlin, Wis.). The sizes of the amplified DNAs were determined by comparison with a 123-bp DNA ladder (GIBCO BRL).

Southern blot or dot blot DNA-DNA hybridizations were performed by using the oligonucleotide probe SHIMA-P. For Southern blots the amplified DNAs in the agarose gel were denatured in a solution containing 0.5 M NaOH and 1 M NaCl for 20 min at room temperature with gentle shaking. The gel was then neutralized in a solution containing 0.5 M Tris-HCl (pH 7.4) and 3 M NaCl for 20 min at room temperature with gentle shaking. The DNAs were then transferred onto a Zetaprobe GT nylon membrane (Bio-Rad Laboratories, Richmond, Calif.) by using 10× SSC (1× SSC is 0.15 M NaCl and 0.015 M trisodium citrate). For dot blot hybridization, the amplified DNAs were denatured by placing the gel in 0.1 volume of a denaturing solution consisting of 0.4 M NaOH and 10 mM Na_2EDTA and boiling the preparation for 10 min in a boiling water bath. The samples were plunged into ice to cool and transferred onto Zetaprobe GT nylon membranes by using a BioDot apparatus (Bio-Rad).

For hybridization, each membrane containing DNAs was treated with hybridization solution consisting of 5× SSC, 20 mM NaH_2PO_4 (pH 7.0), 7% sodium dodecyl sulfate (SDS), 10× Denhardt's solution (1× Denhardt's solution contains 0.2% type V bovine serum albumin, 0.2% polyvinylpyrrolidone, and 0.2% Ficoll), and 100 μg of phenol-chloroform (1:1, vol/vol)-extracted denatured salmon sperm DNA (Bio-Rad) per ml at 65°C for 0.5 h with gentle shaking. The hybridization solution was discarded, and fresh hybridization solution was added to the membrane along with 200 to 300 ng of 3′-end-radiolabelled SHIMA-P probe (the specific activity of the radiolabelled probe was 1×10^7 to 5×10^8 cpm/μg of DNA). Hybridization was performed at 65°C for 4 h with gentle shaking. Following hybridization, the membranes were washed three times with washing buffer I containing 3× SSC, 10× Denhardt's solution, 5% SDS, and 25 mM NaH_2PO_4 (pH 7.5) at 55°C for 10 min each time. The membranes were then washed with washing buffer II containing 1× SSC and 1% SDS, first at 55°C for 5 min and then at 60°C for 3 min with gentle shaking. The membranes were blotted between two Whatman filter papers and wrapped with plastic wrap, and autoradiography was performed with Kodak X-AR film for 2 to 36 h at −75°C.

Specificity of *Salmonella* detection. To determine the specificity of the DNA amplification and detection system, DNA was recovered from *Salmonella* and non-*Salmonella* strains by growing cells on appropriate growth media. The cells were harvested, and total genomic DNA was purified for DNA amplification by using the procedure described by Ausubel et al. (5) to test the specificity of the PCR for *Salmonella* detection. A total of 43 strains and serogroups of *Salmonella* spp. and 97 non-*Salmonella* bacterial species were used in this study to test the specificity of the *himA* gene amplicon (Table 1).

PCR amplification with oligonucleotide primers SHIMA-L and SHIMA-R generated 0.122-kbp amplified DNA bands for all *Salmonella* spp. tested in this study (Fig. 1A). Radiolabelled oligonucleotide probe SHIMA-P produced positive hybridization signals with amplified DNAs from all of the *Salmonella* spp. tested in this experiment (Fig. 1B). No amplification was observed with non-*Salmonella* bacterial strains, including members of the *Enterobacteriaceae*. Thus, PCR amplification in which primers SHIMA-L and SHIMA-R primers were used coupled with gene probe detection with probe SHIMA-P appeared to detect *Salmonella* species specifically.

***Salmonella* detection in oysters.** Oysters were obtained from commercial Gulf of Mexico coastal shellfishermen and transported to a laboratory by using the standard procedures described by the American Public Health Association (1). The oysters were transferred to aquaria containing artificial seawater (Instant Ocean; Aquarium, Mentor, Ohio) and maintained at 15 ± 2°C.

For analysis, the oysters were shucked and homogenized in a Waring blender. Multiple 1.0- and 2.2-g homogenized meat samples from each oyster were suspended in 2.0 and 5.0 ml of sterile water, respectively. Some samples were analyzed both by plating for viable count determinations and by extracting DNA for PCR detection. In some experiments, prior to the PCR and plate count analyses, the oyster meat was incubated in sterile buffered peptone water (23) for 3 h at 37°C with gentle shaking or in sterile Luria-Bertani broth for up to 3 h at 37°C.

To recover DNA for PCR amplification, the homogenized oyster meat samples were treated with guanidine isothiocyanate at a final concentration of 5.9 M, mixed by vortexing, and incubated at 65°C for 90 min. The suspension was diluted with sterile distilled water so that the guanidine isothiocyanate concentration was 0.3 M, mixed by vortexing, and transferred to a boiling water bath for 5 min. The samples were then cooled to room temperature, and sodium acetate was added to a final concentration of 0.3 M. The samples were then centrifuged at 13,000 rpm in a microcentrifuge for 10 min, and the supernatants were transferred to new tubes and extracted twice with an equal volume of chloroform. The DNA was precipitated with 95% ethanol at −20°C and sedimented by centrifugation. The DNA was resuspended in sterile distilled water. Typically, 5 μl of each of the chloroform-purified clear samples was used for PCR DNA amplification. PCR amplification was performed by using primers SHIMA-L and SHIMA-R. The amplified DNA samples were analyzed by agarose gel electrophoresis and by DNA-DNA hybridization in which radiolabelled oligonucleotide probe SHIMA-P was used.

Bacteria in replicate oyster meat samples were also detected by viable plating serial dilutions onto brilliant green agar, bismuth sulfite agar, and/or salmonella-shigella agar to determine the presence of *Salmonella* species and onto Trypticase soy agar (Difco) to determine total viable microbial counts. The incubation times were 24 h.

In some experiments, oysters were artificially exposed to *S. typhimurium*. In these experiments the oysters were starved for 7 days by keeping them at 12°C in a 38-liter aerated holding tank containing artificial seawater (Instant Ocean). The oysters were then transferred to a 4-liter tank for feeding and exposure to *S. typhimurium*. In these tests, *Salmonella* cultures in the late exponential phase were added to achieve final concentrations of 10^1 to 10^4 *Salmonella* cells per ml of seawater, and the oysters were fed 1-ml portions of invertebrate suspension to induce ingestion so that they would accumulate the bacteria in their tissues during the filter-feeding process.

After 1 h of exposure to the *Salmonella*-containing seawater, the oysters were transferred to a separate sterile container, and the viability of the oysters was confirmed by their ability to keep their two shells tightly closed during the shucking process. The outer shells of each of the oysters from seeded and unseeded control tanks were scrubbed with a toothbrush, washed several times with fresh salt water,

TABLE 1. Strains used and PCR detection results

Strain	Result
Acetobacter aceti ATCC 15973	−
Achromobacter xerosis ATCC 14780	−
Acinetobacter calcoaceticus ATCC 15308	−
Aeromonas hydrophila ATCC 7966	−
Alcaligenes denitrificans subsp. *xylosoxydans* ATCC 15173	−
Alcaligenes faecalis ATCC 8750	−
Alteromonas rubra ATCC 29570	−
Arthrobacter citreus ATCC 11624	−
Bacillus cereus ATCC 14579	−
Bacillus subtilis ATCC 6051	−
Bacteroides fragilis ATCC 25285	−
Bdellovibrio bacteriovorus ATCC 15143	−
Beggiatoa alba ATCC 33555	−
Beijerinckia indica ATCC 9039	−
Campylobacter fetus subsp. *fetus* ATCC 27374	−
Campylobacter jejuni ATCC 29428	−
Caryophanon latum ATCC 33407	−
Chromobacterium vinosum ATCC 17899	−
Citrobacter freundii ATCC 8090	−
Clostridium perfringens ATCC 3624	−
Cytophaga johnsonae ATCC 17061	−
Deinococcus radiodurans ATCC 13939	−
Derxia gummosa ATCC 15944	−
Desulfovibrio desulfuricans ATCC 29577	−
Edwardsiella tarda ATCC 15947	−
Enterobacter aerogenes ATCC 13048	−
Enterobacter cloacae ATCC 13047	−
Escherichia coli ATCC 11775	−
Flavobacterium breve ATCC 14234	−
Flavobacterium meningosepticum ATCC 13253	−
Flectobacillus major ATCC 29496	−
Fusobacterium nucleatum ATCC 25586	−
Haemophilus influenzae ATCC 33391	−
Halobacterium saccharovorum ATCC 29252	−
Hyphomicrobium indicum ATCC 19614	−
Klebsiella oxytoca ATCC 12833	−
Klebsiella pneumoniae ATCC 13883	−
Kluyvera ascorbata ATCC 33433	−
Lactobacillus acidophilus ATCC 4356	−
Legionella anisa ATCC 35290	−
Legionella birminghamensis ATCC 43702	−
Legionella cherrii ATCC 35252	−
Legionella cincinnatiensis ATCC 43753	−
Legionella israelensis ATCC 43119	−
Legionella jamestowniensis ATCC 35298	−
Legionella maceachernii ATCC 35300	−
Legionella oakridgensis ATCC 33761	−
Legionella parisiensis ATCC 35299	−
Legionella pneumophila ATCC 33152	−
Legionella pneumophila MICU 33735	−
Legionella pneumophila U7W 33736	−
Legionella rubrilucens ATCC 35304	−
Legionella sainthelensi ATCC 35248	−
Legionella spiritensis ATCC 35301	−
Legionella tucsonensis ATCC 49180	−
Legionella wadsworthii ATCC 33877	−
Leucothrix mucor ATCC 25107	−
Listeria monocytogenes ATCC 15313	−
Micrococcus luteus ATCC 4698	−
Micrococcus roseus ATCC 186	−
Moraxella osloensis ATCC 19976	−
Mycobacterium gordonae ATCC 14470	−
Mycoplasma pneumoniae ATCC 15531	−
Myxococcus fulvus ATCC 25199	−
Neisseria gonorrhoeae ATCC 49226	−
Nitrobacter winogradskyi ATCC 25391	−
Paracoccus denitrificans ATCC 17741	−
Pasteurella caballi ATCC 49197	−
Photobacterium phosphoreum ATCC 11040	−
Proteus mirabilis ATCC 29906	−
Proteus vulgaris ATCC 13315	−
Pseudomonas aeruginosa ATCC 10145	−
Pseudomonas alcaligenes ATCC 14909	−
Pseudomonas cepacia ATCC 25416	−
Pseudomonas diminuta ATCC 11568	−
Pseudomonas fluorescens ATCC 13525	−
Rhizobium meliloti ATCC 9930	−
Rhodopseudomonas blastica ATCC 33485	−
Rhodospirillum rubrum ATCC 11170	−
Salmonella arizonae ATCC 13314	+
Salmonella choleraesuis ATCC 13312	+
Salmonella choleraesuis 8 A 40	+
Salmonella enterica subsp. *bongori* ATCC 43975	+
Salmonella enterica subsp. *diarizonae* ATCC 43973	+
Salmonella enterica subsp. *enterica* ATCC 43971	+
Salmonella enteritidis ATCC 13076	+
Salmonella paratyphi 8 A 39	+
Salmonella paratyphi B DPD 15	+
Salmonella schottmuelleri ATCC 8759	+
Salmonella sp. strain ATCC 35664	+
Salmonella sp. serotype California strain ATCC 23201	+
Salmonella sp. serotype Florida strain ATCC 10727	+
Salmonella sp. serotype Kentucky strain ATCC 9263	+
Salmonella sp. serotype menden strain ATCC 15992	+
Salmonella sp. serotype Minnesota strain ATCC 9700	+
Salmonella sp. serotype Newport strain ATCC 6962	+
Salmonella sp. serotype ochsenzoll strain ATCC 29932	+
Salmonella sp. serotype Phoenix strain ATCC 29931	+
Salmonella sp. serotype Potsdam strain ATCC 25957	+
Salmonella sp. serotype pullorum strain ATCC 19945	+
Salmonella sp. serotype sloterdijk strain ATCC 15971	+
Salmonella sp. serotype Tennessee strain ATCC 10722	+
Salmonella sp. serotype Miami strain GT 58	+
Salmonella sp. serotype Newington strain GT 61	+
Salmonella sp. serotype Newport strain DPD 7	+
Salmonella sp. serotype Newport strain GT 62	+
Salmonella sp. serotype San Diego strain GT 63	+
Salmonella sp. strain SAL A	+
Salmonella sp. strain Senftenberg 8 A 34	+
Salmonella sp. strain SAL C	+
Salmonella thompson DPD 14	+
Salmonella thompson GT 64	+
Salmonella typhi DPD 11	+
Salmonella typhi ATCC 6539	+
Salmonella typhi ATCC 19430	+
Salmonella typhimurium ATCC 14028	+
Salmonella typhimurium ATCC 19585	+
Salmonella typhimurium ATCC 29946	+
Salmonella typhimurium ATCC 13311	+
Salmonella typhimurium ATCC 29629	+
Salmonella typhimurium ATCC 29630	+
Salmonella typhimurium DPD 21	+
Serratia marcescens ATCC 13880	−
Shigella boydii ATCC 35969	−
Shigella boydii DPD 13	−
Shigella dysenteriae ATCC 29026	−
Shigella flexneri ATCC 12022	−
Shigella sonnei ATCC 29930	−
Staphylococcus aureus ATCC 12600	−
Streptobacillus moniliformis ATCC 14647	−
Streptococcus pneumoniae ATCC 6303	−
Streptococcus pyogenes ATCC 12344	−
Streptomyces griseinus ATCC 23915	−
Thermomicrobium roseum ATCC 27502	−
Thermus aquaticus ATCC 25104	−
Thiobacillus intermedius ATCC 15466	−
Vibrio cholerae ATCC 11623	−
Vibrio parahaemolyticus ATCC 17802	−
Yersinia enterocolitica ATCC 9610	−
Zymomonas mobilis ATCC 29191	−

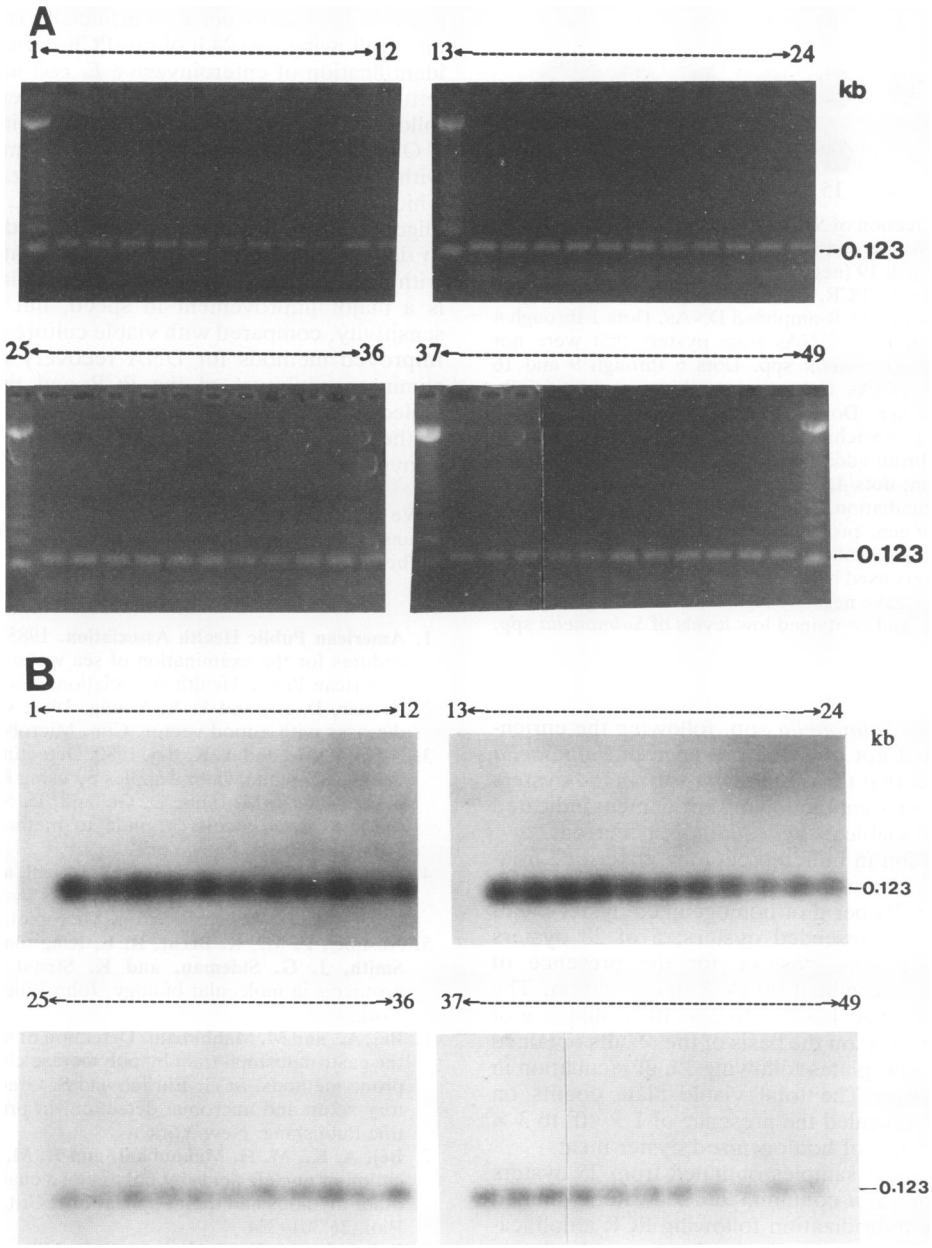

FIG. 1. (A) Agarose gel electrophoresis analyses of the 0.122-kbp amplified DNAs from all of the strains of *Salmonella* spp. listed in Table 1, showing that PCR amplification with primers SHIMA-L and SHIMA-R was positive for all of the *Salmonella* spp. tested. Lanes 1, 13, 25, 37, and 49 contained a 0.123-kbp DNA ladder as size standards; the other lanes contained amplified DNAs from the *Salmonella* spp. listed in Table 1. (B) Southern blot showing hybridization of the amplified 0.122-kbp DNA bands with radiolabelled oligonucleotide probe SHIMA-P. No signal was observed for any of the non-*Salmonella* spp. tested.

and exposed to UV irradiation for 15 min to avoid carryover surface contamination with cells. The oysters were shucked, and the meat was transferred into sterile containers and homogenized by using a Polytron homogenizer for 15 min. The presence of *Salmonella* spp. in the oyster meat was detected by the PCR and viable plate count procedures described above.

PCR detection of *Salmonella* spp. without preenrichment sometimes failed even when plate counts on selective media revealed levels of *Salmonella* spp. as high as 10^2 cells per g of oyster meat. Either inefficient target DNA recovery or interference by unknown factors in the oyster meat led to this lack of direct detection of *Salmonella* spp. by the PCR. Incubation of oyster meat with an enrichment broth for as little as 1 h led to successful PCR detection of *Salmonella* spp. (Fig. 2). At high contamination levels amplification could be detected by both gel electrophoresis and hybridization, but at low contamination levels hybridization was necessary to provide sufficient sensitivity for detection. A 3-h incubation with either buffered peptone water or Luria-Bertani broth resulted in reliable detection of *Salmonella* spp. by the PCR when plate counts yielded a positive result

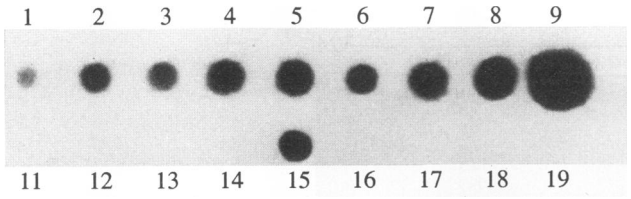

FIG. 2. Dot blot detection of *Salmonella* spp. from oysters. Dots 5 and 15 contained positive controls of *Salmonella* DNA. Dots 11 through 14 and 16 through 19 (negative signals) contained extracted DNAs not subjected to the PCR. Dots 1 through 4 and 6 through 9 (positive signals) contained PCR-amplified DNAs. Dots 1 through 4 and 11 through 14 contained DNAs from oysters that were not artificially exposed to *Salmonella* spp. Dots 6 through 9 and 16 through 19 contained DNAs from oysters that were artificially exposed to *Salmonella* spp. Dots 1, 6, 11, and 16, samples at zero time (before addition of enrichment broth); dots 2, 7, 12, and 17, samples shortly after broth addition; dots 3, 8, 13, and 18, samples 1 h after broth addition; dots 4, 9, 14, and 19, samples after 3 h of preenrichment broth incubation. The results clearly demonstrate the enhanced sensitivity of gene probe detection following PCR and the increase in the PCR signal following preenrichment. Plate counts indicated that the oysters used in this study were contaminated with *Aeromonas* spp., which gave negative signals in the PCR gene probe detection experiments, and contained low levels of *Salmonella* spp. ($\leq 10^1$ cells per g).

(Fig. 2). Detection of *Salmonella* spp. following the enrichment step established not only the presence of *Salmonella* spp. but also the fact that the *Salmonella* spp. in the oysters were viable. A greater signal following enrichment indicated that reproduction of viable *Salmonella* spp. occurred.

Following incubation in buffered peptone water or Luria-Bertani broth for 3 h, it was possible to detect as few as 1 to 10 *S. typhimurium* cells per g of homogenized oyster meat. In one set of tests of unseeded oysters, 5 of 28 oysters (approximately 18%) were positive for the presence of *Salmonella* spp. as determined by PCR amplification. The positive oysters contained 1×10^1 to 2×10^4 cells per g of homogenized oyster meat on the basis of the results obtained with brilliant green agar plates following 3 h of incubation in buffered pepton water. The total viable plate counts on Trypticase soy agar revealed the presence of 1×10^5 to 3×10^6 microbial cells per g of homogenized oyster meat.

All of the oyster meat samples obtained from 25 oysters exposed to *S. typhimurium*-contaminated seawater exhibited positive gene probe hybridization following PCR amplification. On the basis of the results obtained after viable plating on brilliant green agar, 21 samples (approximately 84%) contained 1 to 10 *S. typhimurium* cells per g of homogenized oyster meat and the remainder contained $>10^1$ *S. typhimurium* cells per g of homogenized oyster meat. The samples which were not seeded with *S. typhimurium* and those that were not enriched did not exhibit any PCR amplification. Virtually identical results were obtained in other sets of experiments, each of which was conducted with 20 oysters exposed to *Salmonella* spp. and 10 control oysters. In these experiments *Salmonella* spp. were difficult to detect unless a preenrichment step that allowed reproduction was included; when the preenrichment step was included, the sensitivity of PCR detection was equivalent to 1 to 10 CFU/g of oyster meat.

Our PCR-based detection of *Salmonella* spp. by using preenrichment and DNA extraction was virtually identical to the approach used by Hill et al. (23) to detect *V. vulnificus*. In the study of Hill et al. (23), *V. vulnificus* was detected at a level of 10^2 cells per g of artificially contaminated oyster meat following 4 to 24 h of pre-PCR enrichment. PCR-based identification of enteroinvasive *E. coli* and *Shigella* spp. in lettuce (25) and ground beef (18) has likewise been reported following 6 to 24 h of culturing of cells in broth media.

Our results suggest that PCR DNA amplification coupled with gene probe DNA-DNA hybridization methods, in which oligonucleotide primers SHIMA-L and SHIMA-R and oligonucleotide probe SHIMA-P are used, has the capability to detect *Salmonella* spp. in contaminated oyster samples with high specificity and sensitivity within 3 to 5 hours. This is a major improvement in speed, but not necessarily in sensitivity, compared with viable culture detection methods. Improved methods for DNA recovery and purification to eliminate inhibitors of the PCR and the development of molecular methods for determining viability would represent further improvements for PCR detection of *Salmonella* spp. in oysters.

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