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Evaluation of Hybridization Characteristics of a Cloned pRF106 Probe for *Listeria monocytogenes* Detection and Development of a Nonisotopic Colony Hybridization Assay

CHANGMIN KIM,[1] LEWIS M. GRAVES,[1] BALASUBRAMANIAN SWAMINATHAN,[1]*
LEONARD W. MAYER,[2] AND ROBERT E. WEAVER[1]

Meningitis and Special Pathogens Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,[1] and Division of Vector-Borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado 80522[2]

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An internal fragment (pRF106 fragment, ca. 500 bp) of a gene (*msp*) coding for a 60-kDa protein of *Listeria monocytogenes* serotype 1/2a was used to develop a screening method to discriminate between *L. monocytogenes* and avirulent *Listeria* spp. on primary isolation plates. The *L. monocytogenes*-derived probe fragment of pRF106 hybridized to a 13-kb fragment of *L. monocytogenes* and a 3-kb fragment of one cheese isolate strain of *Listeria seeligeri* under stringent hybridization conditions (mean thermal denaturation temperature [T_m] − 5°C). The probe also hybridized to a 6-kb fragment of *Listeria innocua*, *Listeria ivanovii*, and *L. seeligeri* under less stringent hybridization conditions (T_m − 17°C). The pRF106 fragment was labeled with digoxigenin-11-dUTP and used to develop a colony hybridization assay. Colonies from lithium chloride-phenylethanol-moxalactam agar were blotted onto nylon membranes. The cells were pretreated with microwaves before lysis with sodium hydroxide. DNA-DNA hybridization and posthybridization washing were done at high stringency (T_m − 7°C). The nonisotopic colony hybridization procedure was specific for *L. monocytogenes* when evaluated against pure cultures of *L. monocytogenes* and other *Listeria* species, excluding the cheese isolate of *L. seeligeri*. Also, it was specific for *L. monocytogenes* when evaluated with *Listeria*-negative food enrichment cultures that were inoculated in the laboratory with *Listeria* species.

Interest in the development of rapid diagnostic methods for *Listeria monocytogenes* increased dramatically after the three food-borne listeriosis outbreaks in North America between 1981 and 1985 and the recent Swiss outbreak (1, 9, 23, 33). Nucleic acid probe-based tests appear promising for the rapid and specific detection and confirmation of *L. monocytogenes* in foods (2, 20, 29, 30). A commercially available filter hybridization test uses a DNA probe to a *Listeria* spp.-specific sequence in the rRNA and is genus specific (20). Such a test is useful for quality control testing of foods but is not specific for *L. monocytogenes*, as is needed for use in investigations by regulatory and public health agencies. A colony hybridization test based on a cloned probe (pRF106 fragment) derived from the chromosomal DNA of *L. monocytogenes* was successfully used for the specific detection of *L. monocytogenes* by researchers at the U.S. Food and Drug Administration (4). The Food and Drug Administration colony hybridization assay uses a ^{32}P-labeled probe, which presents problems of safety, license, and disposal for the end user (10).

We adapted the colony hybridization test to a nonisotopic format to eliminate problems associated with the use of radioisotopes. Probe DNA was labeled with digoxigenin-11-dUTP by a random primed DNA labeling method (5). The labeled probe was evaluated in dot hybridization and colony hybridization formats. Hybridization conditions were selected on the basis of data obtained with the ^{32}P-labeled pRF106 *Listeria* chromosomal DNA insert (pRF106 fragment) and were optimized for nonisotopic hybridizations by additional experiments so that *L. monocytogenes* could be differentiated from *Listeria innocua*, other *Listeria* spp., and other bacteria.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. The bacteria strains used in this study are listed in Table 1. Bacterial cultures were stored frozen in sheep blood at −70°C. For revival, the frozen cultures were thawed, grown on blood agar (tryptic soy agar plus 5% sheep blood) at 35°C for 16 to 18 h, and checked for purity before use. *Brochothrix thermosphacta* was grown at 25°C.

Extraction of chromosomal DNA. Chromosomal DNA was extracted from *L. monocytogenes*, other *Listeria* spp., and other gram-positive bacteria by a method developed in our laboratory (11). A cell pellet from an overnight culture in 10 ml of brain heart infusion broth was resuspended in 1 ml of prelysis buffer (0.05 M Tris [pH 7.0], 0.031 mg of pancreatic lipase per ml, 0.3 mg of sodium taurocholate per ml, 0.005 M calcium chloride, 0.2 g of sucrose per ml, 0.005 g of lysozyme per ml) and incubated at 37°C for 45 min. For *Staphylococcus aureus*, 100 U of lysostaphin (Sigma Chemical Co., St. Louis, Mo.) was added to the prelysis buffer (6). Nine milliliters of lysis buffer (0.01 M Tris [pH 8.0], 0.0025 M EDTA [pH 8.0], 2% sodium dodecyl sulfate [SDS], 0.05 mg of proteinase K per ml) was added, and the mixture was incubated at 55°C for 30 min. DNA was purified by phenol and chloroform extractions, precipitated with 95% cold ethanol, and washed with 70% ethanol. DNA was dried in a vacuum and resuspended in 100 μl of deionized water.

Preparation of filters for dot blots. The protocol of Kafatos et al. (18) was used to denature DNA and to immobilize denatured DNA on nylon filters (Magnagraph; pore size, 0.45 μm; Micron Separations, Westboro, Mass.). Chromo-

TABLE 1. Bacterial strains used in the study

No.	Bacterial species	Serotype	EIL[a] No.	Source[b]
1	*Listeria monocytogenes*	1/2a	F4263	Patient
2	*L. monocytogenes*	1/2b	F4260	Patient (blood)
3	*L. monocytogenes*	1/2b	F4233	Patient
4	*L. monocytogenes*	4b	F4243	Patient (CSF)
5	*L. monocytogenes*	4b	F4244	Patient (CSF)
6	*L. monocytogenes*	4b	F4262	Patient
7	*L. monocytogenes*	4b	F4264	Patient
8	*L. monocytogenes*	4b	F4393	Cheese
9	*Listeria innocua*		F4078	ATCC 33090
10	*L. innocua*		F4247	Food isolate from FDA
11	*L. innocua*		F4248	Food isolate from FDA
12	*Listeria murrayi*		F4076	ATCC 25401
13	*L. murrayi*		F4077	ATCC 25402
14	*Listeria seeligeri*		F4080	ATCC 35968
15	*L. seeligeri*		F4088	Cheese
16	*L. seeligeri*		F4856	Beef
17	*L. seeligeri*		F4879	Cheese
18	*L. seeligeri*		F4880	Cheese
19	*L. seeligeri*		F4881	Cheese
20	*L. seeligeri*		F4882	Cheese
21	*L. seeligeri*		F5761	Lunchmeat
22	*Listeria ivanovii*		F4081	ATCC 19119
23	*Listeria welshimeri*		F4082	H. P. R. Seeliger (SLCC 53330)
24	*L. welshimeri*		F4083	ATCC 35897
25	*Listeria grayi*		F4085	ATCC 19120
26	*Jonesia denitrificans*		F4087	ATCC 14870
27	*Brochothrix thermosphacta*		F5950	ATCC 11509
28	*Erysipelothrix rhusiopathiae*		F5937	Patient (blood)
29	*Corynebacterium diphtheriae*		F5944	University of Louisville, Louisville, Ky.
30	*Corynebacterium aquaticum*		F5942	Patient (CSF)
31	*Corynebacterium striatum*		F5946	Patient (blood)
32	*Corynebacterium minutissimum*		F5940	Patient
33	*Bacillus cereus*		F5939	ATCC 6051
34	*Staphylococcus aureus*		D1233	Patient
35	*Serratia fonticola*		F4547	ATCC 29844
36	*Escherichia coli*		D0145	Patient

[a] Epidemic Investigation Laboratory, Meningitis and Special Pathogens Branch, Centers for Disease Control.
[b] CSF, Cerebrospinal fluid; FDA, Food and Drug Administration.

somal DNA was diluted to 200 μg/ml of 0.001 M EDTA. Five microliters of DNA was transferred to a 1.5-ml microcentrifuge tube, and an equal volume of 0.8 M sodium hydroxide was added. The mixture was incubated at 37°C for 10 min, diluted with 90 μl of deionized water, and neutralized with 100 μl of 2 M ammonium acetate. The denatured DNA was loaded on the nylon membrane by using a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Richmond, Calif.). The membrane was dried at room temperature and baked at 80°C for 1 h. The nylon membrane was stored at 4°C until use.

Preparation of filters for colony blots. Colonies of bacteria grown on lithium chloride-phenylethanol-moxalactam (LPM) agar were transferred to nylon filters by overlaying the filter on the agar surface and leaving it there for 3 min. The cells were pretreated with the microwave procedure of Datta et al. (4) before conventional alkaline lysis (12). Denatured DNA was immobilized on the nylon filters by the procedure described above.

Preparation of filters for Southern blots. Chromosomal DNAs (2 μg) of *L. monocytogenes* and other *Listeria* spp. were restricted with *Eco*RI following the manufacturer's instructions (New England BioLabs, Beverly, Mass.). Restricted DNA was electrophoresed on 1.0% agarose (Bethesda Research Laboratories, Gaithersburg, Md.; molecular biology grade) in Tris-acetate buffer at 80 V for 4 h. The gel was stained with ethidium bromide and photographed. DNA fragments were transferred from the gel to nylon membranes by Southern blotting (25).

Isolation of the pRF106 plasmid. *Escherichia coli* LE392 (24) containing the pRF106 plasmid (4, 8) (kindly provided by R. K. Flamm, Washington State University) was grown in L broth, and plasmid was amplified with chloramphenicol (25). Plasmid DNA was isolated by the procedure of Ish-Horowicz and Burke (16) and purified by cesium chloride-ethidium bromide density gradient centrifugation (25).

^{32}P labeling of the pRF106 fragment. pRF106 was digested with *Hin*dIII and *Hin*cII, and the fragments were separated by agarose gel electrophoresis. The pRF106 fragment (500 bp) was recovered from the gel by using a GeneClean kit (Bio 101, La Jolla, Calif.). The pRF106 fragment was labeled with [α-^{32}P]dCTP by nick translation (Bethesda Research Laboratories).

Digoxigenin-dUTP labeling of pRF106 fragment. Labeling was done by using digoxigenin-11-dUTP (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and a random primed DNA labeling method (5, 15). Digoxigenin-labeled DNA was

recovered by ethanol precipitation, resuspended in deionized water, and stored at −20°C until needed.

Hybridization. The T_m of *Listeria* DNA at various monovalent cation (Na^+) concentrations was calculated by the following equation (28): $T_m = 16.6 \log M + 0.41(\%G+C) + 81.5$, where M is the Na^+ concentration and T_m is the mean thermal denaturation temperature. *Listeria* DNA has a G+C content of 37% (34). Hybridization conditions were adjusted to high stringency (T_m − 5°C for the ^{32}P-labeled pRF106 fragment and T_m − 7°C for the digoxigenin-labeled pRF106 fragment) or low stringency (T_m − 17°C) by manipulation of one or more of the following: temperature of hybridization, formamide concentration in the hybridization solution, sodium ion concentration in wash solution, and temperature of washing. Formamide was assumed to lower T_m by 0.72°C per 1% (27). Hybridization of the ^{32}P-labeled pRF106 fragment with target DNA was done at 37°C for 16 to 18 h in 50% formamide–6× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate [0.165 M Na^+])–1× Denhardt solution (0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone)–0.01 M EDTA–0.5 μg of probe (5×10^6 dpm/μg) (T_m − 23.6°C). Posthybridization washing was done in 0.1× SSC–0.1% SDS solution at 50°C for low stringency washing (T_m − 17°C) and at 62°C for high stringency washing (T_m − 5°C). Hybridization of target DNA with the digoxigenin-labeled pRF106 fragment was done at 60°C for 16 to 18 h in 5× SSC–0.1% *N*-lauroylsarcosine–2% SDS–0.5% blocking agent (Boehringer Mannheim)–290 ng of digoxigenin-labeled probe DNA. After hybridization, the membranes were rinsed twice with 2× SSC–0.1% SDS at room temperature. Posthybridization washing was done twice at 60°C for 20 min in 0.1× SSC–0.1% SDS solution. After posthybridization washing, immunologic detection of the hybrid complex was done by following the manufacturer's protocols.

Evaluation of the digoxigenin-labeled pRF106 fragment with artificially contaminated enrichment broths. *L. monocytogenes* (4b) F4393, *Listeria seeligeri* F4880, *L. innocua* F4247, and *Listeria ivanovii* F4081 were inoculated in brain heart infusion broths and incubated at 35°C for 16 to 18 h. The *Listeria* species grown overnight were centrifuged, and cell pellets were diluted to 3×10^4 per ml by using 0.01 M phosphate-buffered saline. The diluted cells (0.1 ml) were inoculated into 1 ml of U.S. Department of Agriculture secondary enrichment broths (26), which were used for isolation of *L. monocytogenes* from contaminated foods and were culturally confirmed as *L. monocytogenes* negative. Samples (100 μl) of the inoculated broths were plated on LPM agar plates, and plates were incubated at 35°C for 48 h. Colony blotting and hybridization with the digoxigenin-labeled pRF106 fragment were done as described above.

RESULTS

Specificity evaluation of pRF106 fragment by dot blots. When hybridization was done at low stringency (T_m − 17°C), the ^{32}P-labeled pRF106 fragment hybridized to six of six strains of *L. monocytogenes*, *L. innocua*, *L. ivanovii*, and *L. seeligeri* (Fig. 1A). No cross-reactivity outside the genus *Listeria* was observed. Under stringent hybridization conditions, the pRF106 fragment hybridized only to six of six strains of *L. monocytogenes* (Fig. 1B). However, when seven strains of *L. seeligeri* were included in the dot blot evaluation (Fig. 2), the pRF106 fragment hybridized strongly to the DNA from a cheese isolate of *L. seeligeri* (F4882) even under stringent hybridization conditions.

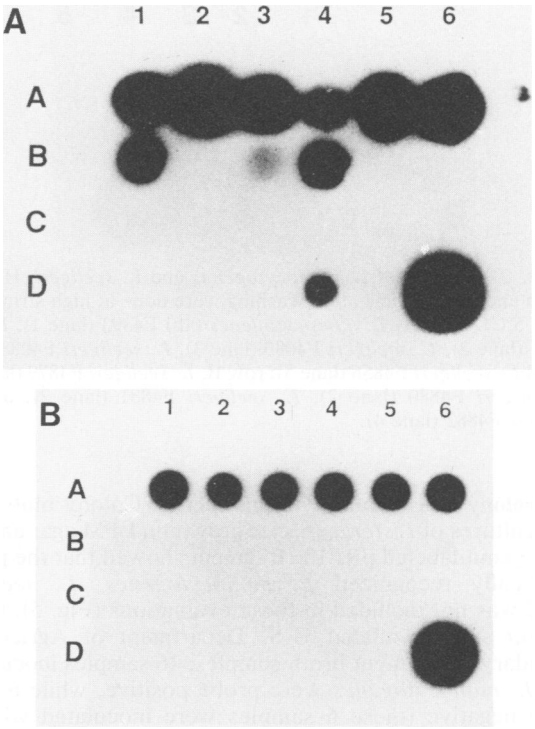

FIG. 1. Dot blots of *Listeria* spp. and other bacteria probed with the ^{32}P-labeled pRF106 fragment. In panel A, hybridization and posthybridization washing were done at low stringency (T_m − 17°C); in panel B, hybridization and posthybridization washing were done at high stringency (T_m − 5°C). (A and B) Rows A, *L. monocytogenes* (1/2a) F4263 (lanes 1), *L. monocytogenes* (1/2b) F4260 (lanes 2), *L. monocytogenes* (4b) F4243 (lanes 3), *L. monocytogenes* (4b) F4244 (lanes 4), *L. monocytogenes* (4b) F4262 (lanes 5), and *L. monocytogenes* (4b) F4264 (lanes 6); rows B, *L. innocua* F4247 (lanes 1), *Listeria murrayi* F4077 (lanes 2), *L. seeligeri* F5761 (lanes 3), *L. ivanovii* F4081 (lanes 4), *Listeria grayi* F4085 (lanes 5), and *Listeria welshimeri* F4083 (lanes 6); rows C, *Jonesia denitrificans* F4087 (lanes 1), *B. thermosphacta* F5950 (lanes 2), *Erysipelothrix rhusiopathie* F5937 (lanes 3), *Corynebacterium diphtheriae* F5944 (lanes 4), *Corynebacterium aquaticum* F5942 (lanes 5), and *Corynebacterium striatum* F5946 (lanes 6); rows D, *Corynebacterium minutissimum* F5940 (lanes 1), *B. cereus* F5939 (lanes 2), *S. aureus* D1233 (lanes 3), *L. seeligeri* F4856 (lanes 4), *E. coli* D0145 (lanes 5), and pRF106 (lanes 6).

Hybridization targets of the pRF106 fragment. Under low stringency hybridization conditions (T_m − 17°C), the pRF106 fragment hybridized to a 13-kb *Eco*RI fragment of *L. monocytogenes* chromosomal DNA, a 6-kb *Eco*RI fragment of *L. innocua*, *L. ivanovii*, and *L. seeligeri*, and a 3-kb *Eco*RI fragment of two strains of *L. seeligeri* (Fig. 3A). When hybridization was done at high stringency (T_m − 5°C), the pRF106 fragment hybridized only to the 13-kb *Eco*RI fragment of *L. monocytogenes* and a 3-kb *Eco*RI fragment of one strain of *L. seeligeri* (cheese isolate, F4882) (Fig. 3B).

Specificity evaluation of the digoxigenin-labeled pRF106 fragment. The digoxigenin-labeled pRF106 fragment hybridized only with *L. monocytogenes* (six of six strains) and with one of two *L. seeligeri* strains when hybridization was done at T_m − 7°C (Fig. 4). Increasing the stringency of hybridization to T_m − 5°C did not eliminate the reaction with *L. seeligeri* F4882.

Evaluation of the digoxigenin-labeled pRF106 fragment

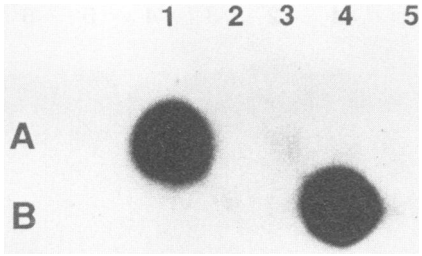

FIG. 2. Dot blot of *L. monocytogenes* and *L. seeligeri*. Hybridization and posthybridization washing were done at high stringency (T_m − 5°C). Row A, *L. monocytogenes* (4b) F4393 (lane 1), *E. coli* D0145 (lane 2), *L. seeligeri* F4080 (lane 3), *L. seeligeri* F4088 (lane 4), and *L. seeligeri* F4856 (lane 5); row B, *L. seeligeri* F4879 (lane 1), *L. seeligeri* F4880 (lane 2), *L. seeligeri* F4881 (lane 3), and *L. seeligeri* F4882 (lane 4).

with colony blots from LPM agar plates. Colony blots with pure cultures of *Listeria* species grown on LPM agar and the digoxigenin-labeled pRF106 fragment showed that the probe specifically recognized *L. monocytogenes*. *L. seeligeri* F4882 was not included in these evaluations (Fig. 5). Of 22 *Listeria* spp.-inoculated U.S. Department of Agriculture secondary enrichment broth samples, 16 samples inoculated with *L. monocytogenes* were probe positive, while 6 were probe negative (these 6 samples were inoculated with *L. seeligeri*, *L. innocua*, and *L. ivanovii*).

DISCUSSION

Digoxigenin labeling is an attractive alternative to radioisotopically labeled probes for several reasons. The labeling protocol is simple and safe. Separation of labeled DNA from unreacted label is easily achieved by ethanol precipitation (13, 15, 32). The label does not appear to affect hybridization characteristics of the probe, and in this study, digoxigenin labeling of the pRF106 fragment did not alter the specificity of the probe. After immunologic reaction, the results were clear-cut and easily interpretable. We encountered no background problems even after 24 h of color development with the substrate. Also, it is possible to reuse the digoxigenin-labeled probe for several hybridizations by following a protocol supplied by the manufacturer.

Flamm et al. (8), in a limited evaluation using four strains (*Bacillus cereus*, *Bacillus thuringiensis*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*), found that the pRF106 fragment does not react with gram-positive bacteria outside the genus *Listeria*, even at a low stringency of hybridization. Datta et al. (4) reported that pRF106 does not hybridize with *Rhodococcus equi*, *S. aureus*, *Streptococcus pyogenes*, and *Streptococcus agalactiae*. The stringency of hybridization used by Datta et al. (4) corresponds to T_m − 30°C. We evaluated the pRF106 fragment against eight gram-positive bacteria outside the genus *Listeria*. We found that the pRF106 fragment does not hybridize to DNA from *B. thermosphacta*, which is closely related to *Listeria* spp. by rRNA cataloging (17). Taken together, the observations of three independent investigators show that the pRF106 fragment does not cross-react outside the genus *Listeria*.

Our observations regarding the specificity of the pRF106 fragment within the genus *Listeria* are in agreement with those of Flamm et al. (8). These authors reported that the pRF106 fragment hybridized to *Eco*RI fragments (7 kb) of *L. innocua*, *L. seeligeri*, and *L. ivanovii* when hybridization

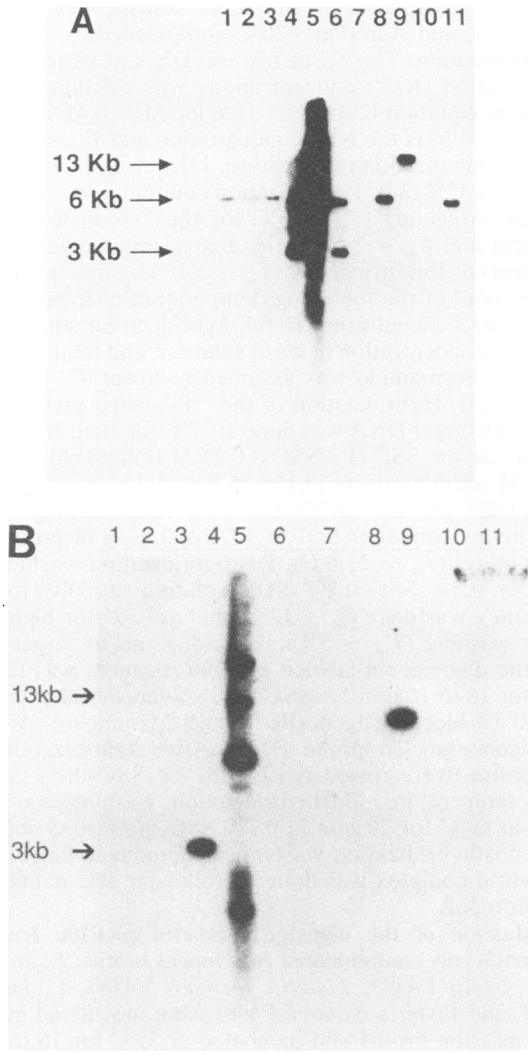

FIG. 3. Southern blots of *Eco*RI-digested *Listeria* chromosomal DNA probed with ^{32}P-labeled pRF106 fragment. In panel A, hybridization and posthybridization washing were done at low stringency (T_m − 17°C); in panel B, hybridization and posthybridization washing were done at high stringency (T_m − 5°C). (A and B) Lanes 1, *L. seeligeri* F4879; lanes 2, *L. seeligeri* F4880; lanes 3, *L. seeligeri* F4881; lanes 4, *L. seeligeri* F4882; lanes 5, uncut pRF106; lanes 6, *L. seeligeri* F4856; lanes 7, *L. murrayi* F4076; lanes 8, *L. innocua* F4078; lanes 9, *L. monocytogenes* (1/2b) F4233; lanes 10, *Serratia fonticola* F4547; and lanes 11, *L. ivanovii* F4081.

was done under nonstringent conditions and hybridized to a 13-kb *Eco*RI fragment of *L. monocytogenes* only under stringent hybridization conditions. The stringent and nonstringent hybridization conditions used by Flamm et al. (8) correspond to T_m − 7°C and T_m − 36°C, respectively, determined by using the formula described by Meinkoth and Wahl (28). Datta et al. (4) evaluated pRF106 under nonstringent hybridization conditions (calculated by us to be T_m − 30°C) and reported that the probe reacted specifically with beta-hemolytic *L. monocytogenes*. Our data and those of Flamm et al. clearly indicate that stringent hybridization conditions need to be used to render the pRF106 fragment specific for *L. monocytogenes*. In addition, Datta et al. (4) reported that the pRF106 fragment does not hybridize to

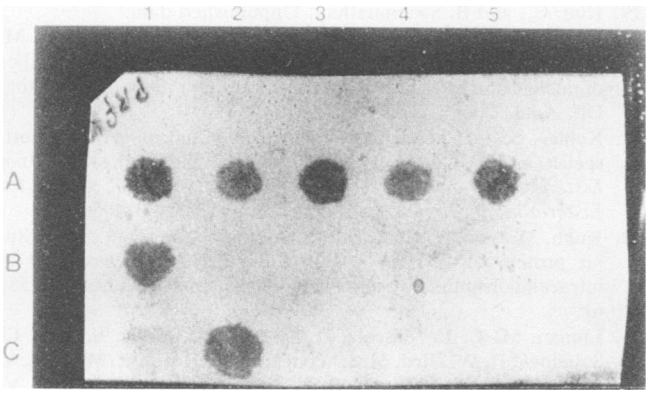

FIG. 4. Dot blots of *Listeria* spp. probed with digoxigenin-labeled pRF106 fragment. Row A, *L. monocytogenes* (1/2a) F4263 (lane 1), *L. monocytogenes* (1/2a) F4260 (lane 2), *L. monocytogenes* (4b) F4393 (lane 3), *L. monocytogenes* (4b) F4244 (lane 4), and *L. monocytogenes* (4b) F4262 (lane 5); row B, *L. monocytogenes* (4b) F4264 (lane 1), *L. innocua* F4247 (lane 2), *L. innocua* F4248 (lane 3), *L. murrayi* F4076 (lane 4), and *L. murrayi* F4077 (lane 5); row C, *L. seeligeri* F4856 (lane 1), *L. seeligeri* F4882 (lane 2), *L. welshimeri* F4082 (lane 3), *L. welshimeri* F4083 (lane 4), and *L. grayi* F4085 (lane 5).

hemolysin-negative *L. monocytogenes* isolated from pasteurized milk and Italian soft cheese (seven strains; Food and Drug Administration reference nos. DA15 and DA17-DA22). When these isolates were biochemically characterized at the Centers for Disease Control, six were found to be *L. innocua* and one was not a *Listeria* spp. strain. Heisick et al. (14) used the pRF106 fragment under nonstringent hybridization conditions (T_m − 45°C) to probe for *Listeria* species in fresh vegetables and in milk from uninfected or infected cows. Their comparison of four methods showed that probing with the pRF106 fragment was the most efficient

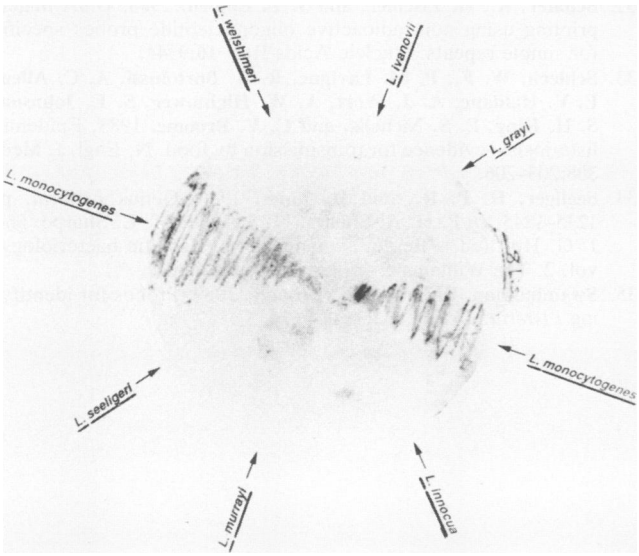

FIG. 5. Colony blots with pure cultures of *L. monocytogenes* and other *Listeria* species on an LPM agar plate probed with the digoxigenin-labeled pRF106 fragment. Strains used were *L. monocytogenes* (4b) F4393, *L. welshimeri* F4082, *L. ivanovii* F4081, *L. grayi* F4085, *L. monocytogenes* (4b) F4234, *L. innocua* F4248, *L. murrayi* F4076, and *L. seeligeri* F4879.

method for detecting *Listeria* species and that the pRF106 fragment probe was significantly better than other *Listeria* spp.-specific methods (*Listeria* spp.-specific DNA probe direct to rRNA from Gene-Trak and an immunoassay using *Listeria* spp.-specific monoclonal antibody from Organon Teknika, Durham, N.C.). Therefore the claims by Datta (3) that the pRF106 fragment probe is specific for *L. monocytogenes* especially under nonstringent hybridization conditions cannot be substantiated and have been challenged (35).

We report for the first time that even under stringent hybridization conditions, the pRF106 fragment reacts with a cheese isolate of *L. seeligeri* (F4882). The 3-kb *Eco*RI restriction fragment of *L. seeligeri* F4882 recognized by the pRF106 fragment is different from the 6-kb *Eco*RI restriction fragments of *L. innocua*, *L. seeligeri*, and *L. ivanovii*. Because the function and role in virulence of the gene product of the pRF106 fragment or its parent fragment containing a complete gene are unknown, it is difficult to speculate on the significance of this finding. However, *L. seeligeri* F4882 does not appear to possess the gene for beta-hemolysin (19) and is avirulent in mice (31). The pRF106 fragment is an internal *Hin*dIII-*Hin*cII fragment of a 5.2-kb *Eco*RI-*Sal*II fragment of *L. monocytogenes* chromosomal DNA originally cloned in plasmid pRF102. The gene product of the pRF102 fragment (*msp*) is not well characterized. Initially, *msp* was thought to code for hemolysin of *L. monocytogenes* (7). However, after the beta-hemolysin (listeriolysin O) gene was cloned and sequenced by Mengaud et al. (29), it was clear that *msp* did not code for beta-hemolysin of *L. monocytogenes*. Subsequent unsubstantiated suggestions have been made that *msp* may be coding for a different hemolysin or possibly for a lipase or a protease that could lyse erythrocytes (8). Recently, Kuhn and Goebel (22) reported that a 60-kDa polypeptide may be involved in the uptake of *L. monocytogenes* by nonprofessional phagocytic cells. It is possible that *msp* may code for the 60-kDa polypeptide involved in invasion, and this hypothesis is currently under investigation.

In conclusion, we have shown that a digoxigenin-labeled pRF106 fragment can be used in a colony hybridization assay for the detection of *L. monocytogenes*. The digoxigenin-labeled pRF106 fragment detected all 16 samples artificially inoculated with *L. monocytogenes*. The digoxigenin-labeled pRF106 fragment worked effectively, was easy to prepare and use, and overcame several disadvantages associated with radioisotopically labeled probes. However, recognition of the specificity characteristics of the pRF106 fragment, particularly as they relate to stringency of hybridization, is essential for proper interpretation of data. The user must recognize that the pRF106 fragment can be used as a *Listeria* spp.-specific or *L. monocytogenes*-specific probe by altering the stringency of hybridization or posthybridization washing. It must also be remembered that certain strains of *L. seeligeri* can hybridize to the pRF106 fragment even at stringent hybridization conditions.

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ADDENDUM

Recently published data (21) suggest that the pFR106 fragment is an internal fragment of the gene coding for an invasion-associated protein (*iap*) in *L. monocytogenes*.

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