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Detection of Hemolytic *Listeria monocytogenes* by Using DNA Colony Hybridization

ATIN R. DATTA,* BARRY A. WENTZ, AND WALTER E. HILL

Division of Microbiology, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, D.C. 20204

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A fragment of about 500 base pairs of the β-hemolysin gene from *Listeria monocytogenes* was used to screen different bacterial strains by DNA colony hybridization. The cells in the colonies were lysed by microwaves in the presence of sodium hydroxide. Of 52 different strains of *Listeria* species screened, only the DNA from beta-hemolytic (CAMP-positive) strains of *L. monocytogenes* hybridized with this probe.

Several recent outbreaks of listeriosis (3, 4) have underscored the lack of rapid methods to isolate and identify *Listeria monocytogenes* from suspected food sources (8; Joseph Lovett, personal communication). Current enrichment schemes may require up to 28 days to recover and characterize cultures of *L. monocytogenes*. Therefore, development of a quick, reliable method would be of great importance. Colony hybridization with radiolabeled DNA probes has been used to rapidly detect and enumerate specific microorganisms in food samples without the need for lengthy enrichment schemes (10). A similar approach should also be useful in identifying *L. monocytogenes*.

L. monocytogenes frequently elaborates an extracellular β-hemolysin called listeriolysin (1, 6, 16). Recent evidence indicates that this β-hemolysin (β-listeriolysin), which is different from α-listeriolysin (14), may be involved in intracellular survival and growth; it also plays a role in producing virulence in the mouse model (5, 12). Therefore, the β-hemolysin gene or some part of it might serve as a genetic probe to detect virulent *L. monocytogenes* strains.

An internal *Hin*dIII-*Hin*cII region of about 500 base pairs of a presumptive β-hemolysin gene of *L. monocytogenes* 10403S (cloned in pUC8) was obtained from Robert Flamm. When used as a gene probe against DNA extracted and purified from *Listeria* spp., this fragment was specific for strains elaborating β-hemolysin, as detected by the CAMP test (a specific test in which a β-lysin-producing *Staphylococcus aureus* strain is used to identify group B streptococci and listeriae) (2).

Crude DNA, extracted from strains of *Listeria* species (Table 1) by acetone, lysozyme, and sodium dodecyl sulfate treatment (9) followed by extraction with phenol-chloroform, provided preparations that were satisfactory as targets when they were affixed to nitrocellulose or nylon filter supports (A. R. Datta, R. K. Flamm, B. A. Wentz, M. F. Thomashow, and W. E. Hill, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, P28, p. 279). This procedure was not suitable for processing homogenized foods, however, since debris apparently prevents the samples from adhering to the supports by filtration. Also, bacteria could be neither easily enumerated nor recovered by this method. Therefore, a colony hybridization technique suitable for *Listeria* strains was developed.

To be practical for routine use in identifying and enumerating beta-hemolytic strains of *L. monocytogenes* isolated from food, clinical, and environmental samples, a colony hybridization method must be available. Unfortunately, in the present study, when colonies on filters were prepared by conventional methods (7, 13), strains of this microorganism failed to give detectable hybridization signals. Treatment of colony filters with acetone, mutanolysin, osmotic agents (sodium chloride and sucrose), or detergents (sodium dodecyl sulfate and Triton X-100) were unsuccessful (data not shown). However, lysozyme (20 mg/ml) treatment followed by treatment with 0.1% sodium dodecyl sulfate yielded marginally successful results (data not shown).

For colony hybridization, the bacterial strains listed in Table 1 were grown overnight at 37°C in brain heart infusion or Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.6% yeast extract and then were spotted in a regular array onto agar plates of the same medium. After overnight incubation at 37°C, filter replicas were made onto filter paper (no. 541, 8.5 cm in diameter; Whatman, Inc., Clifton, N.J.). Filters prepared in this way may be stored at −20°C until they are used. Filters were then irradiated in a microwave oven in the presence of the lysing mixture (1.5 N NaCl in 0.5 N NaOH). Filters "cooked" for 30 s at the high-power setting in a Kenmore microwave oven (model 99471; Sears, Roebuck, and Co., Chicago, Ill.) yielded reproducible results.

The chloramphenicol-amplified plasmid DNA (pRF106) was purified from *Escherichia coli* LE392 by cesium chloride-ethidium bromide density gradient centrifugation. The approximately 500-base-pair β-hemolysin probe was obtained by digesting pRF106 first with *Hin*dIII and then with *Hin*cII, followed by agarose gel electrophoresis and electroelution of the fragment. After the probe was labeled with $dCT^{32}P$ by using a nick translation kit (Bethesda Research Laboratories, Gaithersburg, Md.), hybridization was carried out overnight at 37°C in 50% formamide by using 1×10^6 cpm per filter followed by two 1-h washes at 65°C in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate. Autoradiograms were exposed overnight at −70°C.

The autoradiogram (Fig. 1a) shows the lack of hybridization signal after hybridization with *L. monocytogenes* strains when the colony lysis method of Maas was used (13). However, exposure to microwaves for 30 s resulted in stronger signals being produced by the specifically bound radiolabeled DNA probe (Fig. 1b). Longer exposure times did not improve the quality of the signal (data not shown).

* Corresponding author.

TABLE 1. Bacterial strains used in this study

Strains	Source(s)	Year strain was isolated	Response to CAMP test[a]	Response to probe test[b]
Listeria monocytogenes				
10403S			S+ R−	+
Scott A	Human outbreak	1983	S+ R−	+
DA15	Pasteurized milk	1986	S− R−	−
DA17	Italian soft cheese	1986	S− R−	−
DA18	Italian soft cheese	1986	S− R−	−
DA19	Italian soft cheese	1986	S− R−	−
DA20	Italian soft cheese	1986	S− R−	−
DA21	Italian soft cheese	1986	S− R−	−
DA22	Italian soft cheese	1986	S− R−	−
V-7[c]	Raw milk outbreak	1983	S+ R−	+
V37-CE[c]	Raw milk outbreak	1983	S+ R−	+
SE31	French Brie	1986	S− R−	−
SE35	French Brie	1986	S+ R+	+
Brie 1	French Brie	1986	S+ R−	+
Brie 18	French Brie	1986	S+ R−	+
BS1	French Brie	1986	S+ R−	+
ATCC 15313	Rabbit		S+ R−	+
Murray B	Human outbreak	1983	S+ R−	+
Listeria murrayi				
ATCC 25401	Corn leaves		S− R−	−
ATCC 25402	Corn leaves		S− R−	−
Listeria ivanovii				
ATCC 19119	Sheep		S− R+	−
LA29	French Brie	1985	S− R+	−
LA30	French Brie	1985	S− R+	−
Listeria seeligeri				
BK14	French Brie with pepper		S− R−	−
BK15	French Brie with pepper		S− R−	−
Brie 21	French Brie	1986	S− R−	−
AT2	French Brie	1986	S− R−	−
LA15	Mexican style cheese		S− R−	−
ATCC 35967	Plant environment Soil		S− R−	−
LA43	French Brie	1986	S− R−	−
Brie 34	French Brie	1986	S− R−	−
Brie 35	French Brie	1986	S− R−	−
Brie 42	French Brie	1986	S− R−	−
Brie 59	French Brie	1986	S− R−	−
LA44	French Brie	1986	S− R−	−
Listeria innocua				
2498A	Raw milk		S− R−	−
C194	French Brie	1986	S− R−	−
LA1	Mexican style cheese		S− R−	−
2478KA	Raw milk outbreak		S− R−	−
Listeria grayi				
ATCC 19120	Feces		S− R+	−
ATCC 25400	Corn leaves		S− R−	−
Listeria spp.				
CEB1831	Meat		S+ R−	+
CEB1885	Human placenta		S+ R−	+
CEB1890	Human blood		S+ R−	+
CEB1911	Human		S+ R−	+
CEB1918	Meat		S+ R−	+
CEB1919	Meat		S+ R−	+
CEB1921	Meat		S+ R−	+
CEB2015	Silage		S+ R−	+
CEB2018	Silage		S+ R−	+
CEB2638	Cheese		S+ R−	+
CEB2776	Bovine placenta		S+ R−	+

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TABLE 1—*Continued*

Strains	Source(s)	Year strain was isolated	Response to CAMP test[a]	Response to probe test[b]
Rhodococcus equi				−
Staphylococcus aureus				−
Streptococcus pyogenes ATCC 19615			S+	−
Streptococcus agalactiae			S+	−
Escherichia coli				
JM105(pRF102)			S− R−	+
LE392(pRF106)			S− R−	+
JM83(pUC8)			S− R−	−
HB101			S− R−	−
Vibrio cholerae 2194C			S+	−
Vibrio parahaemolyticus SE-2 (KP+) clinical			S+	−

[a] CAMP tests were performed on sheep blood agar plates with *Staphylococcus aureus* (S) and *Rhodococcus equi* (R).
[b] Results indicate hybridization (+) or no hybridization (−) with the probe, as described in the text.
[c] Strains V-7 and V37-CE are from the Centers for Disease Control, Atlanta, Ga., via Jan Hunt.

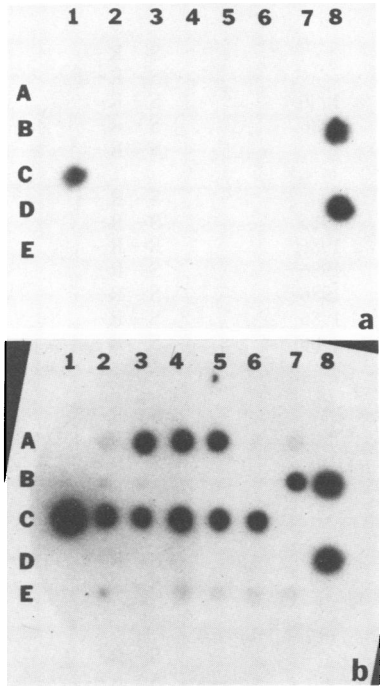

FIG. 1. Autoradiograms of identical arrays of colonies of *Listeria* spp. and other bacteria after colony hybridization with the hemolysin gene probe. The filter in panel a was prepared by the Maas method (13), and the filter in panel b was prepared by using microwaves for irradiation instead of steaming. A2 and A7, *E. coli* HB101; A3 to A5, *L. monocytogenes* 10403S; A6, B2, and B3, *Listeria ivanovii* ATCC 19119; B4 to B6, *L. monocytogenes* DA21; B7, C2, and C3, *L. monocytogenes* V7; B8, C1, and D8, *E. coli* LE392(pRF106); C4 to C6, *L. monocytogenes* ATCC 15313; C7, D1, and D2, *Streptococcus agalactiae* ATCC 13813; D4 to D6, *Vibrio cholerae* 2194C; D7, E2, and E3, *L. ivanovii* LA29; E4 to E7, *E. coli* JM83(pUC8).

This technique should be useful in colony hybridization studies involving other gram-positive bacteria.

The bacterial strains screened with this method, their sources, and the results with the CAMP test and the probe are compared in Table 1. Of 18 *L. monocytogenes* isolates, 10 that were CAMP test (i.e., β-hemolysin) positive were also probe positive (55%). Eleven *Listeria* strains not identified at the species level, which yielded CAMP test-positive results with *Staphylococcus aureus*, were also probe positive. Other *Listeria* species and several hemolysin-producing bacteria belonging to other genera did not hybridize with this probe under stringent conditions. Interestingly, although listeriolysin cross-reacts immunologically with streptolysin O (11, 15), no detectable probe reaction occurred with the β-hemolytic *Streptococcus pyogenes* and *Streptococcus agalactiae* strains.

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