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Detection of *Listeria monocytogenes* by Direct Colony Hybridization on Hydrophobic Grid-Membrane Filters by Using a Chromogen-Labeled DNA Probe

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A DNA probe specific for *Listeria monocytogenes* was isolated from a beta-hemolytic recombinant clone of an *L. monocytogenes* gene bank. It was labeled with horseradish peroxidase and used in a direct colony hybridization method on hydrophobic grid-membrane filters for the detection of the organism. Following color development of the chromogen, a commercial counter (HGMF Interpreter) was able to detect and count the organisms electronically. The method gave a positive reaction with 70 *L. monocytogenes* strains, while showing a negative reaction with 10 strains of other *Listeria* spp. and with 20 organisms of other genera.

Within the past 8 years, five serious outbreaks due to food-borne *Listeria monocytogenes* have been reported in North America and Europe, with mortality rates ranging from 15 to 44% (8). As a result, there has been a marked increase in the number of *Listeria* determinations performed in food microbiology laboratories, and methods which are more rapid and specific than the present conventional ones are required by both regulatory and industrial laboratories (6).

The analytical food microbiology system based on the hydrophobic grid-membrane filter (HGMF) requires that the bacterial growths be colored for detection and identification. The HGMF Interpreter can then be used for rapid, automated counting of the organism of interest (22). Supporting this system, HGMF-based methods that use enzyme-labeled polyclonal and monoclonal antibodies have been developed for the detection of *Salmonella* spp. (1), enterotoxigenic *Staphylococcus aureus* (19), and *Escherichia coli* O157 (24) in foods. Methods that use monoclonal antibodies and DNA probes for bacterial detection are specific and do not require time-consuming biochemical testing for confirmed identification of the organism (13).

The detection of *L. monocytogenes* by DNA colony hybridization in foods was reported by Datta et al., who used as a probe either a fragment from a presumptive beta-hemolysin gene cloned in pUC8 (4) or a synthetic oligonucleotide (5). These probes were labeled with radioisotopes. It seemed that the development of a chromogen-labeled DNA probe specific for *L. monocytogenes* would be useful in a rapid HGMF-based detection method for this organism. We report here on the preparation of a genomic library of *L. monocytogenes* in pUC18 and of its screening for a DNA probe specific for *L. monocytogenes*. The probe was then enzyme labeled and used in a colorimetric colony hybridization method to demonstrate its usefulness in the direct detection of the organism on HGMF.

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MATERIALS AND METHODS

Bacterial strains. *L. monocytogenes* 81-861, serovar 4b, was isolated from the coleslaw implicated in the outbreak of listeriosis in the Maritime Provinces of Canada in 1981. All strains of *Listeria* spp. and of other genera (Tables 1, 2, and 3) were from the collection of the Health Protection Branch or from the American Type Culture Collection. Host cell strain *E. coli* DH5α [F$^-$, *endA1 hsdR17* (r_k^- m_k^+) *supE44 thi-1* λ$^-$ *recA1 gyrA96 relA1* Δ(*argF-lacZYA*)*U169* φ80d*lacZ* Δ*M15*] was obtained from GIBCO/BRL Ltd., Burlington, Ontario, Canada.

Materials. Reagents and equipment were purchased from the following suppliers: restriction endonucleases, T4 DNA ligase, low-melting agarose, supercoiled DNA molecular weight markers, and pUC18 vector DNA were from GIBCO/BRL Ltd.; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), isopropyl-β-D-thiogalactopyranoside, deoxynucleotides, RNase, and ampicillin (sodium salt) were from Boehringer-Mannheim Canada Ltd., Dorval, Quebec; cesium chloride, EDTA, horseradish peroxidase (HRP), sodium dodecyl sulfate (SDS), and polyethylene glycol 8000 were from Sigma Chemical Co., St. Louis, Mo.; Lugalvan G35 (polyethyleneimine) was from BASF Canada, Toronto, Ontario; Gene-Clean was from Bio 101, La Jolla, Calif.; Difco bacteriological media were from BDH Chemicals, Toronto, Ontario; pZ523 spin columns were from 5 Prime→3 Prime Inc., Paoli, Pa.; ISO-GRID HGMF was from QA Laboratories Ltd., Toronto, Ontario; and the HGMF Replicator and HGMF Interpreter System were from Richard Brancker Research Ltd., Ottawa, Ontario.

Media and growth conditions. Stock cultures were maintained at room temperature in a semisolid medium consisting of meat extract (5.0 g), peptone (10.0 g), NaCl (3.0 g), $Na_2HPO_4 \cdot 12H_2O$ (2.0 g), and agar (10.0 g) per liter of medium; the final pH was 7.4. As required, *Listeria* strains were streaked to tryptose agar, and other organisms were streaked to brain heart infusion agar and incubated at 35°C, except for *Brochothrix thermosphacta*, which was incubated at 30°C. Recombinant strains were maintained under selective pressure with ampicillin. Libraries on HGMFs of the 100 organisms listed in Tables 1, 2, and 3 were prepared, replicated, and stored as described in Peterkin et al. (18).

TABLE 1. *L. monocytogenes* strains used for screening the DNA probes

No.	Strain[a]	Serovar	No.	Strain	Serovar
1	V7	1c	66	83-427	4a
2	1472-1	1/2	67	83-641	1/2
3	19112	1/2c	68	83-648	4b
4	19113	3	69	83-649	1/2
5	LA-5	4	70	83-937	4b
6	81-861	4b	71	83-5067	4b
7	19116	4c	72	83-9138	1/2
8	85-362	4d	73	83-10657	1/2
9	19118	4e	74	83-10984	4b
10	82-26	4ab	75	84-13	1/2
41	F4968	4b	76	84-103	1/2
42	F4989	ND[b]	77	84-108	1/2
43	F6861	4b	78	84-109	1/2
44	F7032	ND	79	84-110	1/2
45	19117	4d	80	84-153	1/2
46	DA-2	4	81	84-251	ND
47	DA-3	4	82	84-331	1/2
48	V37CE	ND	83	84-362	1/2
49	Murray B	ND	84	84-434	1/2
50	Scott A	4b	85	84-542	1/2
51	82-129	4b	86	84-607	4b
52	82-130	4b	87	84-2393	ND
53	82-131	4a	88	85-11	4b
54	82-132	4b	89	85-190	4b
55	82-204	1/2	90	85-206	1/2
56	82-335	1/2	91	85-231	1/2
57	82-336	1/2	92	85-232	1/2
58	82-338	1/2	93	85-263	1/2
59	82-339	1/2	94	85-307	1/2
60	82-340	1/2	95	85-416	4d
61	82-341	1/2	96	85-429	1/2
62	82-342	6b	97	85-442	1/2
63	82-464	1b	98	85-492	4b
64	82-465	1a	99	85-496	1/2
65	83-276	4b	100	85-513	1/2

[a] All strains were obtained from the collection maintained at Health Protection Branch, Ottawa, Ontario.
[b] ND, Not determined.

Isolation of chromosomal and plasmid DNA. Chromosomal DNA was prepared by the procedure of Hadley and Deonier (11), with the following modifications. After the preparation of protoplasts, the cell suspension was lysed by the addition of a solution of 50 mM Tris hydrochloride (pH 7.5), 62.5 mM EDTA, 2.5 M LiCl, and 0.4% Triton X-100 (TELT buffer; 2.5 ml/50 ml of culture), with incubation at 35°C for 1 h. After separation in a CsCl density gradient, the recovered chromosomal DNA fraction was further purified by digestion with a solution of proteinase K (200 μg/ml) in 50 mM Tris hydrochloride (pH 8.0), 10 mM EDTA (TE8), and 2.5% SDS, followed by phenol-chloroform extraction. The purified DNA was recovered by ethanol precipitation and dissolved in a suitable volume of TE8.

Plasmid DNA was prepared by an alkaline-detergent lysis

TABLE 2. *Listeria* species other than *L. monocytogenes* used for screening the DNA probes

No.	Organism	Strain	Source[a]
11	*L. innocua*	LA-1	HPB
12	*L. innocua*	29	HPB
13	*L. welshimeri*	160H1-3	HPB
14	*L. seeligeri*	47295μA	HPB
15	*L. seeligeri*	3293	HPB
16	*L. ivanovii*	LA-2	HPB
17	*L. ivanovii*	LA-3	HPB
18	*L. grayi*	1910	ATCC
19	*L. murrayi*	25401	ATCC
20	*L. denitrificans*[b]	14870	ATCC

[a] HPB, Health Protection Branch, Ottawa, Ontario; ATCC, American Type Culture Collection, Rockville, Md.
[b] Reclassified as *Jonesia denitrificans* (21).

TABLE 3. Organisms other than *Listeria* spp. used for screening the DNA probes

No.	Organism	Strain	Source[a]
21	*Erysipelothrix rhusiopathiae*	75-833	HPB
22	*Lactobacillus casei* (rham)	84-121	HPB
23	*Brochothrix thermosphacta*	11 509	ATCC
24	*Corynebacterium aquaticum*	73-958	HPB
25	*Arcanobacterium haemolyticum*	76-763	HPB
26	*Oerskovia* spp.	80-44	HPB
27	*Kurthia zopfil*	33403	ATCC
28	*Escherichia coli*	25922	ATCC
29	*Citrobacter freundii*		HPB
30	*Klebsiella pneumoniae*		HPB
31	*Salmonella johannesburg*		HPB
32	*Enterobacter aerogenes*		HPB
33	*Pseudomonas aeruginosa*		HPB
34	*Aeromonas hydrophila*		HPB
35	*Staphylococcus aureus* (SED)	23235	ATCC
36	*Bacillus cereus*	H30	HPB
37	*Yersinia enterocolitica*	Y28 0:9	HPB
38	*E. coli* DH5α		HPB
39	*Vibrio vulnificus*	2	HPB
40	*Gemella haemolysans*	10379	ATCC

[a] HPB, Health Protection Branch, Ottawa, Ontario; ATCC, American Type Culture Collection, Rockville, Md.

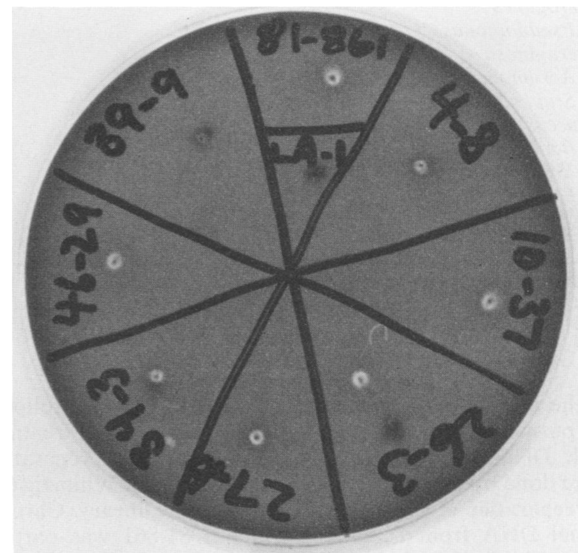

FIG. 1. Zones of beta-hemolysis surrounding recombinant clones after stabbing into 7% horse blood agar and overnight incubation at 35°C. 81-861, *L. monocytogenes* 81-861 (positive control); LA-1, *L. innocua* LA-1 (negative control); 4-8, 10-37, 26-3, 27-6, 34-3, 46-29, and 39-9, isolation numbers of gene bank clones containing recombinant plasmids pIP1, pIP2, pIP3, pIP4, pIP5, pIP7, and pIP6, respectively.

TABLE 4. Comparison of hybridization to colony blots versus direct colony hybridization on HGMF[a]

No. and organism	pIP1		pIP2		pIP3		pIP4		pIP5		pIP6		pIP7	
	CB[b]	HGMF[c]	CB	HGMF	CB	HGMF	CB	HFMB	CB	HGMF	CB	HGMF	CB	HGMF
1. *L. monocytogenes* 1c	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2. *L. monocytogenes* 1/2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3. *L. monocytogenes* 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4. *L. monocytogenes* 3	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5. *L. monocytogenes* 4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6. *L. monocytogenes* 4b	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7. *L. monocytogenes* 4c	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8. *L. monocytogenes* 4d	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9. *L. monocytogenes* 4e	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10. *L. monocytogenes* 4ab	±	±	+	+	+	+	+	+	+	+	+	+	−	+
11. *Listeria innocua*	±	±	+	+	+	+	+	+	±	−	+	−	+	−
12. *Listeria innocua*	±	±	+	±	+	+	±	−	−	−	+	+	+	+
13. *Listeria welshimeri*	−	−	+	+	+	+	+	+	−	−	+	+	+	+
14. *Listeria seeligeri*	+	+	+	+	+	+	+	+	−	−	−	+	+	+
15. *Listeria seeligeri*	−	−	−	−	+	+	+	+	−	−	−	+	+	+
16. *Listeria ivanovii*	−	−	−	−	+	+	+	+	−	−	−	+	+	+
17. *Listeria ivanovii*	−	−	−	−	+	+	+	+	−	−	−	+	+	+
18. *Listeria grayi*	−	−	−	−	+	+	+	+	−	−	−	+	+	+
19. *Listeria murrayi*	−	−	−	−	+	−	+	+	−	−	−	−	−	−
20. *Listeria denitrificans*	−	−	−	−	−	−	−	−	−	−	−	−	−	−
21. *Erysipelothrix rhusiopathiae*	−	−	−	−	−	−	−	−	−	−	−	−	−	−
22. *Lactobacillus casei*	−	−	±	±	+	+	−	−	−	−	−	−	−	−
23. *Brochotrix thermosphacta*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
24. *Corynebacterium aquaticum*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
25. *Arcanobacterium haemolyticum*	−	−	−	−	−	−	−	−	−	−	−	−	−	−
26. *Oerskovia* spp.	−	−	−	−	−	−	−	−	−	−	−	−	−	−
27. *Kurthia zopfii*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
28. *Escherichia coli*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
29. *Citrobacter freundii*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
30. *Klebsiella pneumoniae*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
31. *Salmonella johannesburg*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
32. *Enterobacter aerogenes*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
33. *Pseudomonas aeruginosa*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
34. *Aeromonas hydrophila*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
35. *Staphylococcus aureus*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
36. *Bacillus cereus*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
37. *Yersinia enterocolitica*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
38. *Escherichia coli*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
39. *Vibrio vulnificus*	−	−	−	−	+	+	−	−	−	−	−	−	−	−
40. *Gemella haemolysans*	−	−	−	−	−	+	−	−	−	−	−	−	−	−

[a] Probes were labelled with ^{32}P.
[b] CB, Hybridization to colony blots on Whatman no. 541 paper of colonies grown on tryptic soy agar plates.
[c] HGMF, Direct hybridization to colonies grown on HGMF.

of the cells containing recombinant plasmids (16), followed by purification on a pZ523 spin column (9) and treatment with DNase-free RNase. Rapid, small-scale preparations were done by using the LiCl-boiling method of Wilimzig (25).

Preparation of *L. monocytogenes* genomic library. Chromosomal DNA from *L. monocytogenes* 81-861 was partially digested with restriction endonuclease *Mbo*I (0.25 U/μg of DNA) for 15 min at 37°C. After electrophoresis on a low-melting agarose gel, fragments in the 8- to 12-kbp range were purified with the Gene-Clean kit according to the manufacturer's instructions. Plasmid vector pUC18 DNA (1 μg) was completely restricted with restriction endonuclease *Bam*HI (10 U/μg of DNA). The *L. monocytogenes* chromosomal DNA fragments were ligated to the vector DNA with T4 DNA ligase, at an insert/vector molar ratio of 2. Competent *E. coli* DH5α cells were prepared and transformed with the ligation mixture by the procedure of Hanahan (12). Dilutions of the transformed cells were plated on Luria broth (LB) agar containing 50 μg of ampicillin per ml, 0.004% X-Gal, and 0.01 mM isopropyl-β-D-thiogalactopyranoside. Clones containing recombinant plasmids were screened for beta-hemolytic activity by stabbing cells into blood agar plates (7% horse blood in Columbia agar). After overnight incubation at 35°C, the plates were examined by transmitted light for zones of clearing.

Preparation and labeling of DNA probes. For some exper-

iments, plasmid DNA from beta-hemolytic clones was used as a probe after linearization with restriction endonuclease *Kpn*I. In other cases, after electrophoresis of a *Kpn*I-*Pst*I double digest of plasmid DNA, insert DNA was obtained by cutting the desired fragment from a low-melting agarose gel followed by purification with the Gene-Clean kit. For early screening experiments, probe DNA (linearized plasmid or insert; 0.5 to 1 μg) was radiolabeled with [α-^{32}P]dATP by nick translation to a specific activity of 1.4×10^7 cpm/μg of DNA. For the chromogen labeling, probe DNA was conjugated to HRP by the direct labeling procedure of Renz and Kurz (20). After the cross-linking of HRP to polyethyleneimine, the mixture was concentrated about fourfold by dialysis against polyethylene glycol 8000 and stored at 4°C for up to 6 months without loss of enzyme activity. For labeling, HRP-polyethyleneimine (10 μl) was mixed with probe DNA (≈0.5 μg) and freshly denatured by heating at 100°C for 10 min, followed by rapid chilling on ice. The volume of the HRP-polyethyleneimine-single-stranded DNA probe mixture was made up to 30 μl with 5 mM sodium phosphate, pH 6.8, and 5% glutaraldehyde (6 μl) added. After incubation at 37°C for 10 min, the HRP-labeled probe (36 μl) was added directly to the hybridization solution at the end of prehybridization.

Colony blots on cellulose filters. Colonies grown on tryptic soy agar plates were blotted to a Whatman no. 541 cellulose filter (85-mm diameter) which was then transferred, colony side up, to a filter paper soaked with 0.5 N NaOH–1.5 M NaCl (lysis-denaturation solution). While on the filter paper, the colony blot was exposed to microwave irradiation (≈700 W) for 30 s. It was then transferred, colony side up, to another filter paper soaked with a 0.5 M Tris hydrochloride (pH 7.6)–1.5 M NaCl neutralizing solution, left for 10 min, and then dried for 30 min at ambient temperature. The denatured DNA of the colonies was fixed to the surface of the cellulose filter by UV irradiation (254 nm) at a 10-cm distance for 5 min (100 ergs/mm^2/s). The cellulose filters were prehybridized in 6× standard saline citrate (SSC; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0)–5× Denhardt solution–0.5% SDS at 68°C for 2 h with gentle shaking and hybridized overnight at 68°C in 6× SSC–5 × Denhardt solution containing the heat-denatured ^{32}P-labeled probe DNA (1 μg; 10^6 cpm). The cellulose filters were exposed to X-ray film at −70°C in a cassette fitted with HI$^+$ intensifying screens.

Direct colony hybridization on HGMFs. A master HGMF with 40 organisms (no. 1 to 40 in Tables 1, 2, and 3) was prepared, replicated, and hybridized with the ^{32}P-labeled probes as described in Peterkin et al. (18). Sixty additional *L. monocytogenes* strains (no. 41 to 100 in Table 1) were then added to the master HGMF for testing the chromogen-labeled probe. The master HGMF was replicated and the replicates were incubated as required. The preparation of the HGMF for hybridization was as already described (18). The lysis of the bacterial cells and the denaturation of the DNA were accomplished by exposure of the HGMF carrying bacterial growth to microwave irradiation (≈700 W) for 30 s while it was placed on a filter paper soaked with 0.15 M NaOH in 70% ethanol (2.5 ml; lysis solution). The hybridization procedure was a modification of the method of Downs et al. (7). The treated HGMF, carrying denatured fixed DNA, was prehybridized at 38°C for 2 h in a bag containing a solution of 50% formamide, 2× Denhardt solution, 12% polyethylene glycol 8000, and 0.1% SDS. HRP-labeled DNA probe (36 μl), prepared as described above, was added and hybridization was allowed to proceed overnight at 38°C. The HGMF was washed twice for 15 min at 38°C with vigorous shaking in 50% formamide–1× SSC–0.5% SDS and twice for 15 min at room temperature in 2× SSC. The presence of HRP was detected by placing the HGMF for up to 1 h in a color development solution consisting of 4-chloro-1-naphthol (60 mg) in cold methanol (20 ml) added to 30% hydrogen peroxide (60 μl) in 20 mM Tris hydrochloride (pH 7.5)–500 mM NaCl (100 ml), producing a purple precipitate at the site of the reaction (22).

Statistical analysis. The two detection methods, namely, hybridization to colony blots on cellulose filter papers and direct colony hybridization on HGMFs, were compared at a significance level of $\alpha = 0.05$. The data were first analyzed by probe type, using a χ^2 test, and then the values calculated for the test statistic for each probe type were used to test the combined data (23).

RESULTS

Preparation of *L. monocytogenes* genomic library. *L. monocytogenes* 81-861 chromosomal DNA prepared by the above method yielded 18 μg of DNA per ml of broth culture. It showed an E_{260}/E_{280} ratio of 1.8; there was no evidence for the presence of nucleases active under conditions of restriction digest and of ligation (results not shown). Chromosomal DNA fragments of *L. monocytogenes* were ligated into *Bam*HI-cut pUC18 plasmid vector. The ligation mixture was used to transform *E. coli* DH5α at a transformation frequency of 6×10^6 transformants per μg of DNA. A total of 1.9×10^4 transformants showing insertional inactivation by the production of colorless colonies when plated on medium containing X-Gal were obtained. Agarose gel electrophoresis of DNA from recombinant plasmids showed sizes ranging from 6 to 14 kbp. Assuming an average insert size of 8 kbp, 2,300 transformants gave a 99% probability of cloning all of the chromosomal genes, if the *L. monocytogenes* genome is the same size as that of *E. coli* (3).

Screening of clones for hemolytic activity. Of the 2,500 recombinant clones picked to 7% horse blood agar plates, 7 (0.3%) clones produced zones of clearing indicating beta-hemolytic activity (Fig. 1). The *L. monocytogenes* DNA inserts from these plasmids, named pIP1 to pIP7, had molecular sizes ranging from 490 to 4,140 bp.

Screening recombinant plasmid DNA for *L. monocytogenes* specificity. The development of a procedure which yields good-quality autoradiographs when performing colony hybridizations on HGMFs has been described previously (18). ^{32}P-labeled plasmid DNA from each of the hemolytic clones was screened by colony hybridization against 40 organisms, both by the usual colony blots taken from agar plates and by direct colony hybridization on HGMF (Table 4). There was no evidence of a significant difference ($\alpha = 0.05$) between the results for these two methods of colony hybridization. None of the seven recombinant plasmids, except the DNA of pIP3, hybridized with the 20 organisms of genera other than *Listeria*. The DNA of pIP5 demonstrated good specificity for *L. monocytogenes*, showing sequence homology with the 10 *L. monocytogenes* strains, while hybridizing with none of the other *Listeria* spp. except for weak homology with one strain of *L. innocua* in colony blots, though not on HGMFs.

Chromogen-labeled DNA probe. The DNA of recombinant plasmid pIP5 was now enzyme labeled for further testing as a DNA probe for *L. monocytogenes*. With this HRP-labeled probe, direct colony hybridization under stringent conditions (50% formamide; 38°C) was performed against all 100 organisms (Tables 1, 2, and 3) on HGMFs. The method

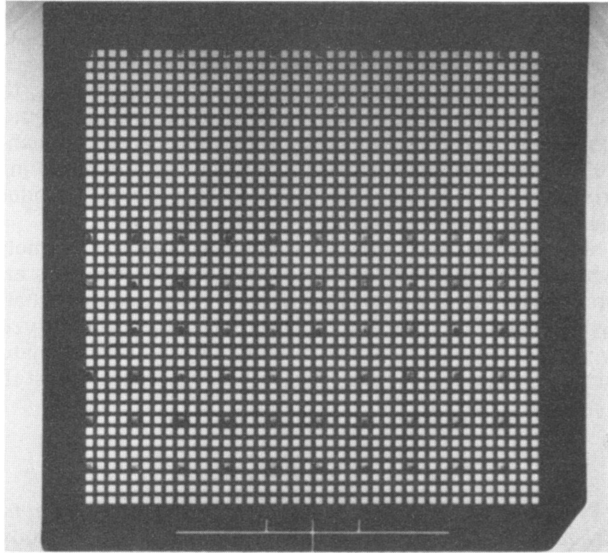

FIG. 2. Appearance of the HGMF containing growths of 100 organisms, 70 of them being *L. monocytogenes* strains, after hybridization with the HRP-labeled DNA probe for *L. monocytogenes* and color development.

showed specificity for *L. monocytogenes*, as sequence homology was demonstrated with the 70 *L. monocytogenes* strains tested, representing all serovars, and no homology was shown with other *Listeria* species or with 20 organisms of other genera (Fig. 2). The probe did not hybridize with other *Listeria* spp. or with organisms of other genera. A 1.4-kbp fragment prepared from a *Kpn*I-*Pst*I double digest of pIP5 DNA was then labeled with HRP in the same way. When tested by colony hybridization on HGMF against the 100 organisms, it gave the same results (not shown).

Automated counting of HGMFs. The HGMFs containing purple grid cells could be easily and accurately read in the HGMF Interpreter (Fig. 3); in the case of the HGMF shown in Fig. 2, the Interpreter yielded a count of 70 ± 1.1.

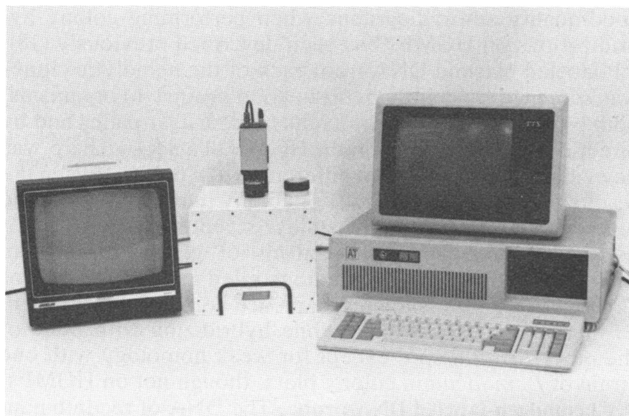

FIG. 3. HGMF Interpreter. The HGMF with stained growths, contained in a petri dish, is inserted under the TV camera and the HGMF image is displayed on the camera monitor. Menu-driven software displayed on the system monitor directs the operator for image detection and analysis.

DISCUSSION

The automation of food microbiology ideally requires methods which are rapid and specific and which allow for electronic data acquisition. The use of enzyme-labeled monoclonal antibodies or DNA probes in colorimetric HGMF methods can fill these requirements. The detection step is rapid and specific compared with conventional methods (13). Furthermore, the growth of interest identified by a colored precipitate can provide an electronic signal by means of an automatic counter such as the HGMF Interpreter (22). As well as this specific example, colorimetric labeling has additional advantages as compared with radioactive labeling, such as shorter detection periods, safer laboratory conditions, and no licencing requirements.

Colorimetric immunological methods are available at present for use with the HGMF (1, 19, 24). These methods are generic in that, given an antibody specific to the test organism and the sample preparation appropriate to the food, the enzyme-labeled antibody protocol can be applied to a wide range of microorganisms. In theory the same holds true for DNA probes, but the use of enzyme-labeled DNA probes in HGMF methods has not yet been explored.

Several radiolabeled DNA probes for the detection of *Listeria* spp., or for *L. monocytogenes* specifically, have been reported (2, 4, 5, 10, 14, 15, 17). Three of the *L. monocytogenes*-specific probes have been used only for Southern blotting and their use has not yet been extended to colony hybridization (2, 10, 15). Notermans et al. (17) developed a radiolabeled DNA probe encoding a delayed hypersensitivity factor which detected *L. monocytogenes* and *L. ivanovii* strains in a colony hybridization method. The only probe reported for food use with a colorimetric label is the commercial probe sold by Gene-Trak Systems (14). However, this has the disadvantage of being genus rather than species specific. As there is no evidence of other species in the *Listeria* genus than *L. monocytogenes* being pathogenic to humans (8), it could be argued that it is preferable to use a DNA probe in foods to detect the pathogenic species rather than to use members of the genus *Listeria* as indicator organisms.

The colorimetric DNA probe method reported here, a direct colony hybridization technique on HGMF, shows no statistical differences from the accepted colony hybridization technique that uses colony blots. The validity of the automated counting by the HGMF Interpreter has been compared previously with manual counting by two analysts on seven types of food (22). It showed no significant method differences at $\alpha = 0.05$. It appears that this HGMF-based method with electronic detection and counting of *L. monocytogenes* may be useful in food microbiology. Testing of the method in food products in the presence of mixed cultures is under way.

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