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A Synthetic Oligonucleotide Probe and a Cloned Polynucleotide Probe Based on the *yopA* Gene for Detection and Enumeration of Virulent *Yersinia enterocolitica*

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We compared a synthetically produced 19-mer oligonucleotide probe with a polynucleotide probe consisting of a cloned fragment of the virulence gene *yopA* for their relative efficiencies in identification and enumeration of virulent *Yersinia enterocolitica*. The probes were used in DNA-DNA colony hybridization assays to differentiate 70 *Yersinia* strains with known plasmid profiles. All 19 strains harboring the 40- to 50-megadalton virulence plasmid were positive in the hybridization assay, whereas their isogenic derivatives lacking this plasmid were negative. Both probes correctly identified plasmid-bearing variants of *Y. enterocolitica* serogroups O:3, O:5,27, O:8, O:9, O:13, and O:21 from three continents. In contrast, none of the probes hybridized with DNA from 32 environmental yersiniae belonging to 26 serogroups not associated with disease. Colony hybridization was used to detect and enumerate virulent *Y. enterocolitica* in three artificially contaminated food samples. Despite a large background of indigenous bacteria (3×10^4 CFU), the efficiency of enumeration ranged from 33 to 82%. The use of nylon filters did not impair the growth of virulent yersiniae. Both probes showed a perfect concordance in their specific differentiation and enumeration of virulent *Y. enterocolitica*. DNA colony hybridization with these two probes permitted rapid and reliable identification of all common pathogenic serogroups without the need for enrichment or esoteric identification protocols.

Yersinia enterocolitica has become the subject of considerable interest in the context of food hygiene (12). Of special significance is its ability to multiply at temperatures approaching 0°C, a circumstance which means that it can grow in properly refrigerated foods (8, 12). *Y. enterocolitica* is considered to be a food-borne pathogen, even though attempts to isolate the bacterium from a suspected food source have seldom proved successful (8).

One can expect to find a broad spectrum of bacteria belonging to several *Yersinia* species and a number of *Y. enterocolitica* serogroups in foods (8, 16). The vast majority of these bacteria have no medical importance whatsoever. Pathogenic significance in humans is associated mainly with a few serogroups (16), and the development of isolation procedures which clearly differentiate pathogenic from nonpathogenic variants has proven problematic. The different serogroups vary in their tolerance to selective components and other factors during the isolation process (8, 26, 31). Variations in tolerance even exist among the different pathogenic serogroups. Because these serogroups show different geographical distributions (16), a method which works well in one part of the world may not necessarily be applicable in other countries. A number of isolation methods are currently in use in different parts of the world, and there is thus great need for a universally acceptable reference method. No one method seems to give an optimal recovery of all the pathogenic serogroups.

All pathogenic serogroups of *Y. enterocolitica* harbor a 40- to 50-megadalton plasmid, which is necessary but not sufficient in itself for expression of virulence (5, 22). Closely related virulence plasmids are found in *Y. pseudotuberculosis* and *Y. pestis* (22). Among the factors encoded by the virulence plasmids is a series of temperature-inducible outer membrane proteins, one of which is designated Yop1 (for *Yersinia* outer membrane protein 1) (3, 23). Experimental studies indicate that Yop1 is a virulence determinant in *Y. enterocolitica* but not in *Y. pseudotuberculosis* (4, 11). The structural gene, *yopA*, encoding a subunit of Yop1, has been cloned and sequenced (2, 27).

The present communication describes the development and testing of two genetic probes: (i) a polynucleotide probe consisting of a restriction endonuclease-generated fragment of the cloned *yopA* gene, and (ii) a synthetically produced 19-mer oligonucleotide probe selected on the basis of sequence analysis of *yopA*. The purposes of the present investigation were (i) to determine the sensitivity and specificity of the probes for identification of virulent *Y. enterocolitica* and (ii) to compare their relative efficiencies for detection and enumeration of virulent *Y. enterocolitica* in artificially contaminated foods.

(A report of this investigation will be included in a dissertation presented by Kari Dommarsnes for the degree of Cand. Scient. at the University of Oslo.)

MATERIALS AND METHODS

Bacterial strains. A total of 70 *Yersinia* strains belonging to 29 serogroups were selected for study (Table 1). Nineteen strains harbored the 40- to 50-megadalton virulence plasmid (18). Each of these strains was represented by plasmid-bearing (P^+) and plasmid-cured (P^-) isogenic derivatives. P^- mutants were derived from P^+ parents by repeated subcultivation on magnesium oxalate agar (18, 21). Strains were characterized with respect to their ability to undergo spontaneous autoagglutination, calcium-dependent growth, mouse virulence, and restriction endonuclease cleavage patterns of the plasmids; all of these experiments are presented

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TABLE 1. *Yersinia* strains examined

Strain	Source	Country	Donor	Serogroup	Biovar[a]	Phagevar	REAP[b]	Filter location[c]	
								P^+	P^-
Y. enterocolitica (pathogens)									
29D-4	Human	Norway	J. Lassen	O:3	4	VIII	1	1	2
Y2	Pig	Norway	T. Nesbakken	O:3	4	VIII	1	3	4
MCH700	Human	Canada	C.-J. Lian	O:3	4	IXb	1	5	6
YE859	Pork	Canada	J. Devenish	O:3	4	IXb	1	7	8
PA11400	Human	Japan	H. Fukushima	O:3	3	II	1	9	10
M388	Pork	Japan	H. Fukushima	O:3	3	II	1	11	12
D113	Dog	Japan	H. Fukushima	O:5,27	2	Xz	2	25	24
YE873	Pork	Canada	J. Devenish	O:5,27	2	Xz	3	26	27
8081	Human	United States	I. Bölin	O:8	1B	Xz	4	28	29
FRI-YE1	Pig	United States	M. P. Doyle	O:8	1B	Xz	5	30	31
NY81-71	Human	United States	T. J. Quan	O:8	1B	Xz	6	32	33
WA	Human	United States	B. Swaminathan	O:8	1B	Xz	7	34	35
1821	Human	United States	M. Shayegani	O:8	1B	Xz	9	36	37
YE653	Human	Canada	J. Devenish	O:8	1B	Xz	8	38	39
YE099	Human	Canada	J. Devenish	O:9	2	X3	10	18	19
PA177	Human	Japan	H. Fukushima	O:9	2	X3	10	20	21
W827	Pig	Belgium	G. Wauters	O:9	2	X3	10	22	23
YE886	Monkey	Canada	J. Devenish	O:13	1B	Xo	11	40	41
YE737	Human	Canada	J. Devenish	O:21	1B	Xz	12	42	43
Y. enterocolitica (nonpathogens)									
8018	Rodent	Norway	This laboratory	O:3	1A				13
Y325	Minced pork	Norway	T. Nesbakken	O:3	4				44
Y377	Minced pork	Norway	T. Nesbakken	O:5	1A				45
Y383	Forced pork	Norway	T. Nesbakken	O:6,30	1A				46
Y362	Forced pork	Norway	T. Nesbakken	O:7,8	1A				47
Y407	Forced pork	Norway	T. Nesbakken	O:10	1A				48
Y381	Pork chops	Norway	T. Nesbakken	O:12	1A				49
Y334	Sausage meat	Norway	T. Nesbakken	O:13	1A				50
Y367	Forced meat	Norway	T. Nesbakken	O:25	1A				51
Y390	Forced meat	Norway	T. Nesbakken	O:41	1A				52
Y393	Minced pork	Norway	T. Nesbakken	O:41,42	1A				53
Y361	Sausage	Norway	T. Nesbakken	O:41,43	1A				54
Y364	Forced meat	Norway	T. Nesbakken	O:44	1A				55
Y347	Forced pork	Norway	T. Nesbakken	NAG[d]	1A				56
Y. frederiksenii									
176-36/80	Sewage	West Germany	S. Aleksic	O:3					14
N2	Fish	Norway	This laboratory	O:4					57
N30	Fish	Norway	This laboratory	O:14					58
Y889	Cooked pork	Norway	T. Nesbakken	O:16					59
Y898	Forced meat	Norway	T. Nesbakken	O:35					60
Y. kristensenii									
Y332	Pork	Norway	T. Nesbakken	O:3					16
Y882	Pig tonsil	Norway	T. Nesbakken	O:11					61
Y891	Forced meat	Norway	T. Nesbakken	O:12					62
Y385	Pork chops	Norway	T. Nesbakken	O:12,25					63
Y883	Pig tonsil	Norway	T. Nesbakken	O:28					64
Y. intermedia									
357-36/85	Water	West Germany	S. Aleksic	O:3					15
Y887	Pig tonsil	Norway	T. Nesbakken	O:4,33					65
Y892	Pork chops	Norway	T. Nesbakken	O:17					66
Y331	Forced pork	Norway	T. Nesbakken	O:21,46					67
Y399	Forced pork	Norway	T. Nesbakken	O:25,26					68
Y360	Forced pork	Norway	T. Nesbakken	O:52					69
Y344	Pork chops	Norway	T. Nesbakken	O:57					70
Y405	Forced pork	Norway	T. Nesbakken	NAG					71

[a] According to the revised biotyping scheme of Wauters et al. (32).
[b] REAP, Different restriction endonuclease cleavage patterns of plasmid DNA (18).
[c] Numbers refer to the autoradiogram in Fig. 1 (position 17 was not inoculated). P^+ and P^- signify plasmid-bearing and plasmid-cured isogenic derivatives, respectively.
[d] NAG, Not agglutinated by available antisera (30).

in a previous communication (18). All the strains were maintained at −70°C in heat-inactivated horse serum with 17% glycerol.

Preparation of bacterial inocula. For examination of pure cultures, the bacteria were grown for 48 h at room temperature (22 to 25°C) on blood agar plates. Bacterial colonies were picked with a sterile inoculation needle and spotted onto nylon membrane filters (NEF-978; Du Pont, NEN Research Products, Boston, Mass.). Filters were placed on blood agar plates, which were subsequently incubated at room temperature overnight. Up to 24 colonies were spotted onto each filter. All strains were examined on the same day, in the same hybridization chamber. For examination of artificially contaminated food, the bacteria were grown overnight at room temperature in tryptic soy broth (Difco Laboratories, Detroit, Mich.) with shaking. The bacterial density was adjusted spectrophotometrically to approximately 10^9 bacteria per ml, and the appropriate inocula were subsequently achieved by serial 10-fold dilutions in peptone-water. The exact number of yersiniae in the inocula was determined by plating 0.1-ml portions of decimal dilutions in duplicate onto cefsulodin-irgasan-novobiocin (CIN) agar plates (CM 653 and SR 109; Oxoid Ltd., Basingstoke, England) without filters.

Inoculation of food samples. Three food products (minced pork, forced pork, and frikadelle [Table 3]) were purchased from local stores and kept at 4°C for up to 3 h before examination. The foods selected are representatives of common pork products containing different amounts of pork, total protein, and spices. A 10-g portion of each food item was homogenized in 90 ml of peptone-water for 1 min by using a Colworth 400 stomacher (A. J. Seward, London, England). Aerobic plate counts and coliform counts were determined by standard methods (19, 20). Food homogenates were inoculated with a plasmid-bearing variant of *Y. enterocolitica* O:3 (strain Y2 P^+) previously isolated from a pig carcass (17). Portions (1 ml) of bacterial dilutions were added to the blended foods. The inoculated foods were homogenized for an additional 2 min, and 0.1-ml samples of the homogenates (yielding 10^2 to 10^4 yersiniae per ml) were spread onto nylon filters on CIN agar plates in duplicate. The plates were incubated at room temperature for 48 h.

Preparation of filters for colony hybridization. To lyse the bacteria and denature their DNA, filters with bacterial colonies were removed from the agar plates and placed sequentially on three filter papers (no. 3MM; Whatman, Inc., Clifton, N.J.) soaked with (i) 10% sodium dodecyl sulfate (SDS), (ii) 0.5 M NaOH, and (iii) 1.5 M NaCl–0.5 M Tris hydrochloride (pH 8.0), respectively (13). The filters were subsequently air dried, packed in plastic, exposed to UV light (302 nm) for 2 to 3 min for fixing single-stranded DNA to the filter matrix, and stored for up to 5 months before hybridization.

Cloning of the *yopA* gene. The structural gene *yopA* was obtained from the virulence plasmid pYV6471/76 of a human clinical isolate of *Y. enterocolitica* serogroup O:3 (strain 6471/76). The recombinant plasmid pYMS4024 was constructed by cloning *yopA* into pBR322 as described elsewhere (2, 27). Plasmid pYMS4 was derived from pYMS4024 by subcloning a *Cla*I-*Sph*I fragment (27). pYMS4 was used to transform *Escherichia coli* PM191 in which the plasmid was maintained.

Isolation and labeling of the polynucleotide probe. Plasmid pYMS4 was isolated from *E. coli* PM191(pYMS4) by using the alkaline lysis technique of Birnboim and Doly (1). Plasmid DNA was purified by CsCl gradient centrifugation followed by dialysis against distilled water and phenol-chloroform extraction (13). The purified plasmid DNA was digested with the restriction endonuclease enzymes *Pvu*II and *Cla*I under conditions recommended by the manufacturer (Toyobo Co. Ltd., Osaka, Japan). The resulting DNA fragments were separated by electrophoresis for 2 h at 50 V and 4°C on a horizontal gel consisting of 0.7% low-melting-point agarose (Bio-Rad Laboratories, Richmond, Calif.). An 862-base-pair fragment containing a part of the *yopA* gene was excised from the gel and purified by using the commercial GeneClean kit (BIO 101 Inc., La Jolla, Calif.). The fragment concerned is completely internal to the *yopA* gene (27). The purified DNA fragment was labeled by the random primer method (6) by using [^{32}P]dCTP (Amersham International PLC, Buckinghamshire, England) and a commercial kit (RPN.1601Y; Amersham). Labeled fragments were separated from unincorporated nucleotides by running the sample through a Sephadex G-50 spin column (13).

Synthesis and labeling of the oligonucleotide probe. The nucleotide sequence of the *yopA* gene was determined in a previous study (27). On the basis of those results, the following 19-mer nucleotide sequence was selected as a DNA probe: 5′-TGCCGCAGAGACACTGATC-3′. This sequence, designated HW-1, is located in the coding region of the signal peptide (27) and within the *Cla*I-*Pvu*II fragment used as a polynucleotide probe in this study (see above). This sequence was completely conserved within *Y. enterocolitica* serogroups O:3 and O:8, *Y. pseudotuberculosis*, and *Y. pestis* (27). The oligonucleotide was chemically synthesized by using an automatic DNA synthesizer (ABI 381A; Applied Biosystems, Inc., Foster City, Calif.) and labeled at the 5′ end with [^{32}P]ATP (Amersham) by using bacteriophage T4 polynucleotide kinase (Amersham), as described by Maniatis et al. (13). Labeled probes were separated from unincorporated nucleotides by running the sample through a Sephadex G-50 spin column (13).

Colony hybridization with the polynucleotide probe. Before hybridization, the probe was denatured at 100°C for 5 min. Prehybridization and hybridization were carried out at 65°C in a solution containing 5× SSPE (13), 5× Denhardt solution (13), 0.1% SDS, and 100 μg of denatured herring sperm DNA per ml (Boehringer Mannheim GmbH Biochemica, Mannheim, Federal Republic of Germany). Prehybridization was performed for 1 to 2 h, whereas hybridization proceeded for 17 h. Approximately 14 ng of probe DNA with a specific activity of 6.3×10^8 cpm/μg of DNA was added to ca. 80 ml of hybridization fluid. The filters were washed at 65°C successively in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) (13) for 15 min, 2× SSC–0.1% SDS for 30 min, and 0.1× SSC for 10 min.

Colony hybridization with the oligonucleotide probe. Filters were prehybridized at 45°C for 1 h in a solution containing 6× SSC, 5× Denhardt solution, 100 μg of denatured herring sperm DNA per ml, 0.05% sodium PP_i, and 0.5% SDS. Hybridization proceeded at 45°C for 2.5 h in 6× SSC–1× Denhardt solution–0.05% sodium PP_i. Approximately 120 ng of probe DNA with a specific activity of 2.3×10^8 cpm/μg of DNA was added to ca. 80 ml of hybridization fluid. The filters were washed four times for 5 min each at 57°C in 6× SSC–0.05% sodium PP_i. To assess the optimal temperature of stringent washing for the probe, five parallel sets of filters, each spotted with 19 isogenic pairs consisting of plasmid-bearing and plasmid-cured derivatives (Table 1), were hybridized and subsequently washed at 47, 50, 53, 56, and 59°C. Minimal background and no false-negative readings

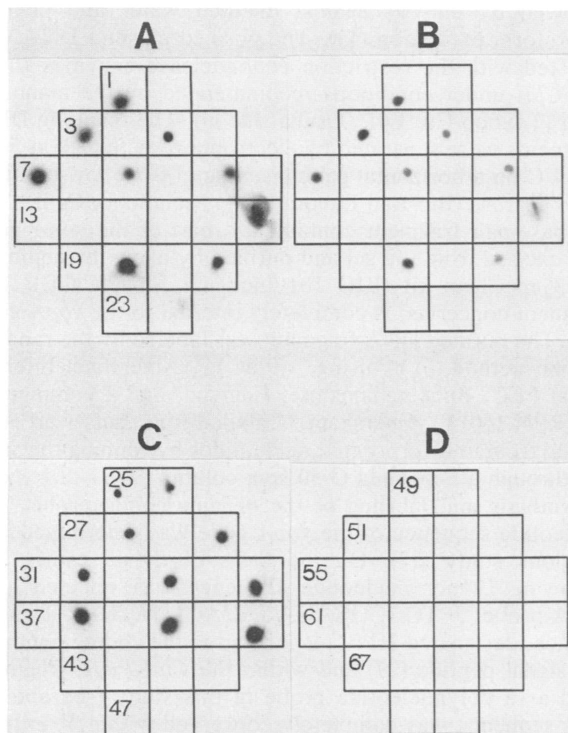

FIG. 1. Autoradiogram of colony hybridization filters inoculated with pure cultures of virulent and avirulent *Yersinia* strains. The strains presented are listed in Table 1 (position 17 was not inoculated). (A, C, and D) Filters hybridized with the polynucleotide probe. (B) The same filter as in panel A hybridized with the oligonucleotide after deprobing.

were obtained at 56 to 59°C. Consequently, all subsequent experiments were performed at 57°C.

Autoradiography. After being washed, the filters were air dried. Autoradiograms were exposed for 1 to 3 days at −70°C by using X-OMAT AR film (Eastman Kodak Co., Rochester, N.Y.) and regular intensifying screens.

Deprobing. All DNA hybridization experiments were first carried out with the polynucleotide probe. To remove the probe, the filters were placed in 0.1× SSC–0.5% SDS at 100°C and cooled to 50°C. They were then hybridized with the oligonucleotide probe. The effectiveness of the deprobing was tested by autoradiography.

RESULTS

Sensitivity and specificity of the probes: examination of pure cultures. The 70 *Yersinia* strains listed in Table 1 were grown in pure culture, inoculated onto blood agar plates with nylon filters, and examined by colony hybridization. Regardless of the type of probe used, all 19 strains harboring the 40- to 50-megadalton virulence plasmid were positive in the hybridization assays, whereas their isogenic plasmid-cured derivatives were negative (Fig. 1). Both probes correctly identified plasmid-bearing variants of *Y. enterocolitica* serogroups O:3, O:5,27, O:8, O:9, O:13, and O:21 isolated from human patients, animals, and foods in Europe, North America, and Japan. The strains examined harbored plasmids with 11 different restriction endonuclease cleavage patterns (18) (Table 1). All of these plasmid variants contained genetic information which rendered their host strains capable of virulent behavior (18). Despite this genetic heterogeneity, all

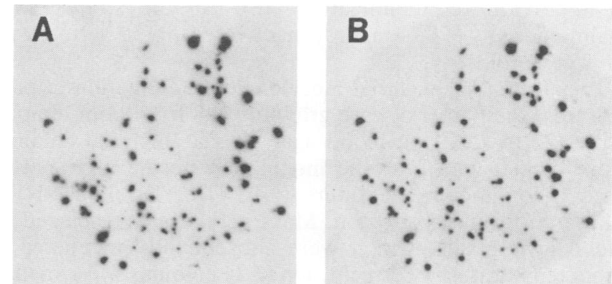

FIG. 2. Autoradiogram of colony hybridization filters inoculated with approximately 10^2 cells of the plasmid-bearing *Y. enterocolitica* strain Y2 P^+ and 10^3 cells of its plasmid-cured derivative Y2 P^-. (A) Filters were hybridized with the polynucleotide probe. (B) Filters were hybridized with the oligonucleotide probe after deprobing.

plasmid-bearing strains were positive in the hybridization assay. Colony hybridization showed perfect concordance with three virulence-associated phenotypic properties encoded by the virulence plasmid: spontaneous autoagglutination, calcium-dependent growth, and mouse virulence (18).

To ascertain whether the probes were able to differentiate pathogenic *Yersinia* strains from nonpathogens, we tested both probes against 32 environmental yersiniae representing a spectrum of serogroups and species. No hybridization was observed with DNA from 14 *Y. enterocolitica* strains belonging to serogroups not associated with disease (Table 1; Fig. 1). All of these strains lacked the virulence plasmid. Likewise, negative results were obtained with 18 strains of *Y. frederiksenii*, *Y. kristensenii*, and *Y. intermedia*, all of which lacked the plasmid concerned. Accordingly, both the sensitivity and specificity of the probes for identification of virulent *Y. enterocolitica* were 100%.

Approximately 10^2 cells of the plasmid-bearing strain Y2 P^+ were mixed with about 10^3 cells of its plasmid-cured derivative (Y2 P^-) and spread on CIN agar plates with filters. Filters were hybridized successively with the poly- and oligonucleotide probes, with intermittent deprobing. No significant difference between the probes in terms of their efficiency in detecting virulent *Y. enterocolitica* could be demonstrated (Fig. 2). The same numbers of spots were observed on the autoradiograms with both probes.

Plating efficiency on nylon filters. To assess whether the presence of nylon filters affected the growth of virulent *Y. enterocolitica*, we spread a suspension of strain Y2 P^+ on CIN agar, blood agar, and MacConkey agar plates with and without filters in triplicate. The number of colonies detected on plates with and without filters did not differ significantly ($P > 0.05$ by Student's two-tailed t test) (Table 2).

Examination of seeded foods. To investigate whether virulent *Y. enterocolitica* could be efficiently detected in foods,

TABLE 2. Plating efficiency on nylon filters

Medium	Nylon filter	No. of colonies[a]			
		Plate 1	Plate 2	Plate 3	Mean
CIN agar	With	23	34	29	29
CIN agar	Without	26	25	30	27
MacConkey agar	With	24	31	27	27
MacConkey agar	Without	23	27	27	26
Blood agar	With	25	21	37	28
Blood agar	Without	22	20	29	24

[a] Colony counts on three parallel plates are indicated.

TABLE 3. Detection and enumeration of virulent *Y. enterocolitica* in artificially contaminated foods by colony hybridization

Food[a]	No. of yersiniae (CFU)			% Recovery
	Expected[b]	Observed[c]		
		Polynucleotide	Oligonucleotide	
Minced pork[d]	0	0	0	
	8	6	6	75.0
	140	115	115	82.1
Forced pork	0	0	0	
	54	44	44	81.5
	>400	>400	>400	
Frikadelle	0	0	0	
	21	7	7	33.3
	195	99	99	50.8

[a] Aerobic plate counts (CFU per gram): 3.2×10^5 (minced pork), 7.0×10^6 (forced pork), 6.3×10^6 (frikadelle); coliform counts (CFU per gram): 3.5×10^2 (minced pork), 4.0×10^3 (forced pork), 2.0×10^5 (frikadelle); plate counts on CIN agar with filter (CFU): not tested (minced pork), 3.5×10^4 (forced pork), 3.0×10^4 (frikadelle). The three rows for each food represent three different inocula.

[b] Determined by spreading the inocula on CIN agar plates without filters. Mean values from three parallel plates are indicated.

[c] Determined by spot count on autoradiograms. Mean values for two parallel filters are indicated.

[d] See Fig. 3.

three food products were inoculated with different concentrations of strain Y2 P^+, spread onto CIN agar plates with filters, and examined by colony hybridization (Table 3; Fig. 3). Despite a large background of indigenous bacteria on the filters (approximately 3×10^4 CFU), 33 to 82% of the added bacteria were detected on the autoradiograms. Again, the poly- and oligonucleotide probes did not differ in their ability to detect virulent yersiniae.

DISCUSSION

There are considerable difficulties associated with the isolation of *Y. enterocolitica* from foods (12, 26, 31). Most methods require time-consuming enrichments to achieve optimal isolation. Moreover, no currently available method allows optimal recovery of all pathogenic serogroups. Developments in gene technology now permit the production of genetic probes which permit rapid detection and enumeration of all pathogenic *Y. enterocolitica* serogroups without the need for an enrichment step (28). Since the probes used in this study were based on a virulence determinant present in all pathogenic variants, they were able to identify a broad spectrum of serogroups from three continents with equal efficiency. In contrast, certain conventional isolation media are known to impair the growth of serogroups O:8 and O:5,27, which prevail in the United States, although they are excellent for the recovery of the serogroups O:3 and O:9, which are commonly encountered in Europe (26, 31). CIN agar, however, permits the growth of all pathogenic serogroups (26). The combination of CIN agar and DNA colony hybridization should therefore be universally acceptable.

The problem with all selective agar media described thus far, including CIN, is that they provide inadequate differentiation between pathogenic *Y. enterocolitica* and other strains. These media allow the growth of a number of *Yersinia* variants to which no medical significance is attributed currently. These nonpathogenic variants are common in many foods, and their colony morphologies make them difficult to distinguish from the pathogens (12). It is important to bear in mind that such environmental yersiniae may conceal the presence of pathogenic variants, with underes-

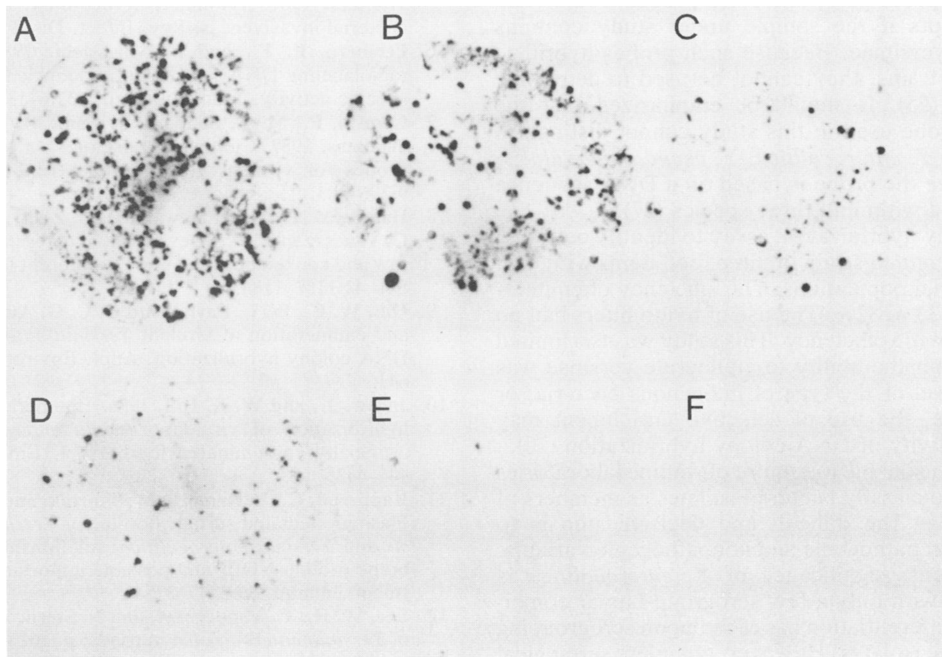

FIG. 3. Autoradiogram of colony hybridization filters inoculated with minced pork experimentally contaminated with various numbers of virulent *Y. enterocolitica* cells (strain Y2 P^+). (A and B) 10^3 cells (two parallel filters); (C and D) 10^2 cells (two parallel filters); (E) 10 cells; (F) food sample before inoculation. Filters were hybridized with the polynucleotide probe (see Table 3).

timation of the latter as a likely consequence. The two DNA probes used in the present study were both able to differentiate pathogenic variants from a spectrum of environmental yersiniae representing 26 serogroups and four species (Table 1).

Both polynucleotide and oligonucleotide probes have been used successfully in previous studies to differentiate virulent and avirulent yersiniae (7, 9, 10, 14, 25). Robins-Browne et al. (25) used polynucleotide probes from eight nonoverlapping regions of the virulence plasmid and from the *inv* and *ail* chromosomal loci responsible for the invasive capacity of *Y. enterocolitica* and *Y. pseudotuberculosis*. Hill and coworkers (9, 10) used a pool of three restriction endonuclease (*Bam*HI) fragments derived from the virulence plasmid pYV8081 of a serogroup O:8 strain to detect and enumerate virulent *Y. enterocolitica* in foods. Recently, Miliotis et al. (14) described a 24-base synthetic DNA oligomer based on nucleotide sequences of the virulence plasmid pYV and used this probe for detection of virulent *Y. enterocolitica* in foods.

Although polynucleotide probes are resistant to loss of sensitivity caused by minor nucleotide mismatches, their specificities should be carefully monitored, and they are difficult to produce in large amounts. Oligonucleotide probes, on the other hand, may be synthesized in large quantities and are highly specific. Their sensitivities, however, are affected by minor nucleotide changes, and the stringency of the hybridization and washing conditions is crucial. Although some discrepancies among different types of probes would not be unexpected, the present study demonstrated complete correspondence between the oligonucleotide and polynucleotide probes. One disadvantage with the present probes is that they are unable to detect potentially pathogenic *Y. enterocolitica* which have accidentally lost the virulence plasmid during the isolation procedure. Although this is not likely to be a common event, this potential problem may be solved by using a chromosomal virulence determinant, for example the *inv* or *ail* loci (15, 25), as a basis for probe selection. Such probes would yield false-positive results if the sample under study contains plasmid-negative yersiniae. Because such probes hybridize with plasmidless strains, they cannot be used to determine virulence per se (25). It should be emphasized that the oligonucleotide probe used in this study cannot distinguish among pathogenic *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*, since the probe is based on a DNA sequence which is conserved within all three species (27).

We used a colony hybridization assay to identify cells of a virulent *Y. enterocolitica* strain in three food items with high indigenous microbial populations. The efficiency of enumeration ranged from 33 to 82%. The use of nylon filters had no significant effect on the efficiency. This study was too limited to ascertain whether the ability to enumerate yersinae was affected by the level or the type of indigenous bacteria, or both. In any case, the use of selective enrichment may increase the sensitivity of DNA colony hybridization (10).

It would seem reasonable to require of routine laboratories that they be able to classify bacterial isolates as members of the genus *Yersinia*. The difficult and decisive step is to distinguish between pathogenic and nonpathogenic variants. Since the pathogenic significance of *Y. enterocolitica* is mainly associated with only a few serogroup-biovar combinations (16), this differentiation has relied upon serogrouping and biotyping of the isolates. However, complete serological and biochemical classification lies outside the scope of most routine laboratories. Furthermore, it would seem pertinent to question the reliability of this identification when used in laboratories with little experience in the identification of yersiniae. A number of different in vitro tests have been proposed for differentiation of pathogenic and nonpathogenic variants (24, 29). Many of these are based on phenotypic properties associated with the virulence plasmid. However, these properties are subject to problems of gene expression in vitro, and the sensitivity and specificity of the tests have been questioned (24, 29). The present results indicate that DNA colony hybridization may permit rapid and reliable identification of all common pathogenic serogroups directly on the primary plate, without the need for esoteric identification protocols. The probes thus offer an alternative to traditional isolation and identification methods. Nonradioactive labeling of suitable probes would make the application of this technique feasible in routine laboratories.

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