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Enumeration by DNA Colony Hybridization of Virulent *Yersinia enterocolitica* Colonies in Artificially Contaminated Food

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A genetic probe was used to identify and enumerate virulent *Yersinia enterocolitica* colonies in 11 artificially contaminated foods. Efficiency of enumeration, determined by autoradiography after DNA colony hybridization, ranged from 66 to 100% (average, 86%) and was influenced by the number of indigenous bacteria. The use of nitrocellulose filters and agar medium had little effect on efficiency of enumeration.

DNA colony hybridization (7) facilitates the examination of food-borne bacterial isolates for the presence of particular genes. Genes for the enterotoxins of *Escherichia coli* have been cloned and used to probe for similar genes in *E. coli* recovered from clinical samples (6, 12–15, 19, 20). We used DNA colony hybridization with a ^{32}P-labeled DNA probe, consisting of *Yersinia enterocolitica* 8081 plasmid DNA gene fragments, to determine the presence of and to enumerate a virulent strain of *Y. enterocolitica* in 11 foods artificially contaminated with this strain. The probe consisted of a pool of fragments (9) or a single cloned fragment from plasmid pDP26 (D. Portnoy, personal communication), encoding the calcium dependency region that is conserved in *Yersinia* species harboring the virulence-associated plasmid (16, 17). Of the seeded *Y. enterocolitica*, 66 to 100% could be detected by autoradiography. Efficiency of enumeration was affected by the indigenous microbial load of the food sample, but not by the medium used or the presence of a nitrocellulose membrane filter.

A culture of *Y. enterocolitica* 8081 (16, 17) was incubated overnight at 26°C in 10 ml of brain heart infusion broth and used to artificially contaminate 11 different foods. A 50-g portion of each food was homogenized in 450 ml of Butterfield buffer by the method described by Andrews (1). The aerobic plate count was determined by diluting and spread plating 0.1 ml on plate count agar as described by Messer et al. (11). The gram-negative plate count was determined by spread plating 0.1 ml of homogenate on MacConkey agar (BBL Microbiology Systems, Cockeysville, Md.) with or without nitrocellulose filters. The CFU titer of an overnight culture of *Y. enterocolitica* 8081 was determined by diluting and spread plating as described above. Samples of this culture were added to the blended food, resulting in dilutions of 10^1 to 10^4 cells per ml. The food was homogenized for an additional 30 s; 0.1-ml samples of the homogenates (yielding 10^0 to 10^3 cells per ml) were spread plated in duplicate on nitrocellulose filters on MacConkey agar. Cultures were incubated at 26°C for 24 to 48 h, depending on colony growth. Colonies on the filters were lysed as described by Hill et al. (8). Because of the high indigenous microbial populations, two of the food products (alfalfa sprouts and mung bean sprouts) were exposed for a few seconds to a 1:25 dilution of 0.5% KOH–0.5% NaCl before being plated on nitrocellulose filters (3). Duplicate filters for each food were prepared and used for hybridization (8, 9). To test the efficiency of enumerating *Y. enterocolitica* 8081 seeded into each food, we compared the number of spots on the autoradiograms (Fig. 1) with the expected number of colonies as determined from the CFU titer.

In seven separate trials, the CFU titers of *Y. enterocolitica* 8081 per gram of food were compared on MacConkey agar with or without nitrocellulose filters. The plating efficiency averaged 94% of that found without filters (Table 1). Microbial loads of the 11 foods tested ranged from 1.2×10^1 to 3.2×10^8 CFU/g on plate count agar; similar counts ($\leq 1 \times 10^1$ to 2.5×10^8 CFU/g) were obtained on MacConkey agar (Table 2). The use of a nitrocellulose filter did not substantially affect the plating efficiency on MacConkey agar ($\leq 1 \times 10^1$ to 2.1×10^8 CFU/g). Autoradiograms of filters from the 11 food samples that had been seeded with approximately 10^2 *Y. enterocolitica* 8081 organisms per ml were used to determine efficiency of enumeration by this method. Sampling errors because of low numbers of seeded cells (≤ 20) produced data that were not usable for bullhead fish and oysters; these results have been omitted. From 66 to 100% (average, 86%) of the input cells were enumerated in the remaining nine foods (Table 3).

The process of isolating and identifying virulent strains of *Y. enterocolitica* from various foods (4, 5, 10, 18, 21–23) is tedious and time-consuming, and virulence tests are not well suited for screening large numbers of isolates (2). However, because DNA colony hybridization does not require isola-

TABLE 1. CFU titer (cells per ml) for *Y. enterocolitica* on MacConkey agar

Trial[a]	CFU titer (cells/ml)		% Recovery[b] with filter
	Without filter[c]	With filter	
1	5.0	6.3	126
2	8.1	8.0	99
3	3.4	2.0	59
4	8.6	8.7	101
5	7.9	7.4	94
6	9.4	8.4	89
7	9.0	8.4	93

[a] One trial was conducted with each food-seeding experiment.
[b] Average recovery ± standard deviation, 94 ± 20%.
[c] Nitrocellulose filter, as described in the text.

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TABLE 2. Aerobic plate counts of foods tested

Food	Plate count on:			Efficiency[a] on filters (%)
	PCA[b]	MacConkey agar (% of PCA)		
		Without filter[c]	With filter	
Celery	2.1×10^5	1.7×10^5 (81)	1.4×10^5 (67)	82
Head lettuce	4.2×10^5	3.1×10^5 (74)	2.4×10^5 (57)	77
Alfalfa sprouts[d]	1.5×10^8	5.9×10^7 (39)	3.3×10^7 (22)	56
Mung bean sprouts[d]	3.2×10^8	2.5×10^8 (78)	2.1×10^8 (66)	84
Oysters	7.9×10^4	8.7×10^3 (11)	6.5×10^3 (8)	75
Bullhead fish	5.4×10^6	3.3×10^5 (6)	1.7×10^5 (3)	52
Rabbit	7.4×10^6	2.6×10^6 (35)	2.2×10^6 (30)	85
Pork	3.0×10^6	6.1×10^4 (2)	5.2×10^4 (2)	85
Tofu	9.4×10^7	1.8×10^5 (0.2)	1.5×10^5 (0.2)	83
Brie cheese	4.3×10^7	2.0×10^5 (0.5)	1.3×10^5 (0.3)	65
Milk	1.2×10^1	$\leq 1 \times 10^1$ (83)	$\leq 1 \times 10^1$ (83)	100

[a] Average efficiency ± standard deviation, 79 ± 14%.
[b] PCA, Plate count agar. Tested without nitrocellulose filters.
[c] Nitrocellulose filters, as described in the text.
[d] Exposed to 1:25 dilution of 0.5% KOH–0.5% NaCl before being plated.

tion of pure cultures (9), it is effective for identifying and enumerating virulent *Y. enterocolitica* strains in a variety of foods with a wide range of microbial loads. On MacConkey agar the number of indigenous microflora is reduced, but *Y. enterocolitica* will proliferate. If the total microbial load was above 3×10^7 CFU/g (unpublished data), identification of *Y. enterocolitica* on MacConkey agar became difficult. The alkali treatment (3), however, enabled us to enumerate *Y. enterocolitica* organisms in the presence of a 10-fold-higher indigenous microbial load.

Identification of the seeded *Y. enterocolitica* was possible after incubation for 24 to 48 h at 26°C. Incubation was continued until growth of indigenous microorganisms in the food was easily observed (average time, 35 h). Although identification was previously made (9) in 48 h by this method, our study showed that in many instances *Y. enterocolitica* could be identified with a shorter incubation period at 26°C. Using DNA colony hybridization, we observed *Y. enterocolitica* at ≤100 CFU/g with a sensitivity of approximately 1 cell per 300,000 indigenous microorganisms. Data indicated that with KOH treatment, selective agars are not necessary until the total aerobic plate count exceeds 10^7 CFU/g (Table 2). These methods can be used together with DNA colony hybridization for identifying *Y. enterocolitica*

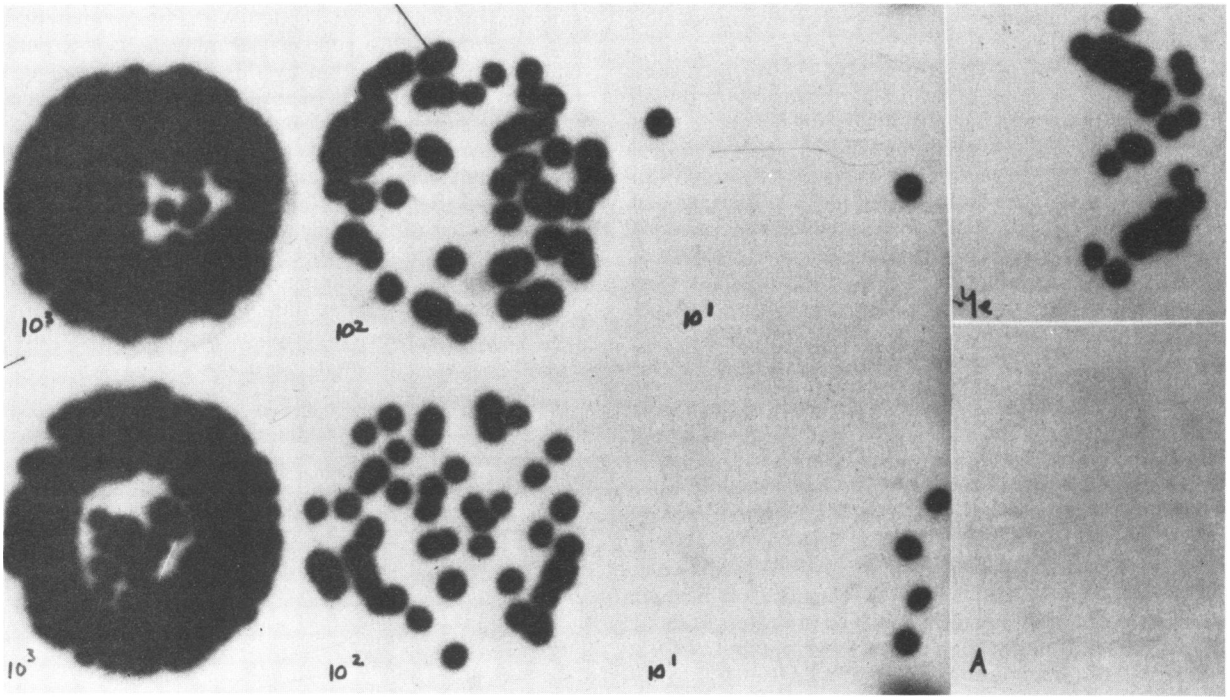

FIG. 1. Autoradiogram of *Y. enterocolitica* colonies from artificially contaminated alfalfa sprouts. Filters were prepared from alfalfa sprouts contaminated with 10^3, 10^2, and 10^1 cells. Right top, *Y. enterocolitica* 8081; bottom right, alfalfa sprouts before the addition of *Y. enterocolitica* 8081.

TABLE 3. Detection of *Y. enterocolitica* 8081 in artificially contaminated food

Food	No. of *Y. enterocolitica* colonies	
	Added/ml[a]	Observed (%)[b]
Celery	63	56 (89)
Head lettuce	63	60 (95)
Alfalfa sprouts	80	57 (71)
Mung bean sprouts	80	53 (66)
Oysters	20[c]	36 (180)
Bullhead fish	20[c]	43 (215)
Rabbit	87	68 (78)
Pork	74	71 (96)
Tofu	84	71 (85)
Brie cheese	84	76 (90)
Milk	84	84 (100)

[a] As determined from CFU titer.
[b] Determined by spot count on autoradiogram. Average ± standard deviation, 86 ± 12%.
[c] Colony number too low to consider results.

in foods with indigenous microbial populations of $\geq 10^7$ CFU/g.

DNA colony hybridization was effective for rapidly enumerating virulent *Y. enterocolitica* strains in a number of foods. Our ability to identify and enumerate these strains was affected by the level of indigenous microflora but not by the type of food. Selective enrichments and plating media increased the sensitivity of DNA colony hybridization.

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