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**Research Note**

# Microarray Analysis Reveals the Actual Specificity of Enrichment Media Used for Food Safety Assessment

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## ABSTRACT

Microbial diagnostic microarrays are tools for simultaneous detection and identification of microorganisms in food, clinical, and environmental samples. In comparison to classic methods, microarray-based systems have the potential for high throughput, parallelism, and miniaturization. High specificity and high sensitivity of detection have been demonstrated. A microbial diagnostic microarray for the detection of the most relevant bacterial food- and waterborne pathogens and indicator organisms was developed and thoroughly validated. The microarray platform based on sequence-specific end labeling of oligonucleotides and the phylogenetically robust *gyrB* marker gene allowed a highly specific (resolution on genus and/or species level) and sensitive (0.1% relative and $10^4$ CFU absolute sensitivity) detection of the target pathogens. In initial challenge studies of the applicability of microarray-based food analysis, we obtained results demonstrating the questionable specificity of standardized culture-dependent microbiological detection methods. Taking into consideration the importance of reliable food safety assessment methods, comprehensive performance assessment is essential. Results demonstrate the potential of this new pathogen diagnostic microarray to evaluate culture-based standard methods in microbiological food analysis.

Foodborne diseases and outbreaks are a major health issue, even in developed countries. For the year 2008, the European Union reported a 5,332 foodborne disease outbreaks resulting in 45,622 human illness cases, 6,230 hospitalizations, and 32 deaths *(5)*. These data indicate a strong need for reliable food safety assessment methods. Standard methods, as defined by the International Organization for Standardization (ISO), are principally culture based. Enrichment procedures are the most salient bottlenecks for reliable target cell detection *(7),* and potential drawbacks of such methods have been reviewed *(9)*. In this study, we used a microarray-based approach to assess the performance of enrichment media for the detection of foodborne pathogens. The evaluation also addressed the influence of product native microflora on the outcome of the analysis.

## MATERIALS AND METHODS

All experiments were performed with artificially contaminated food samples. Various strains of four common food pathogens, *Listeria monocytogenes* (strains SLCC 2755, NCTC 5105, and NCTC 7973), *Salmonella* (*Salmonella* Typhimurium DSM 544 and *Salmonella* Enteritidis DSM 9898), *Campylobacter* (*C. jejuni* DSM 4688 and *C. coli* DSM 4689), and *Yersinia enterocolitica* (NCTC 10460) were inoculated in food matrices of epidemiological relevance (e.g., meat, egg, cheese).

For each experiment, multiple sample sets were prepared by triplicate inoculation of up to four different levels of a single pathogen (0, 1 to 10, 10 to 100, and 100 to 1,000 CFU) in 25 g of each food sample. Bacterial cultures used for spiking were prepared as follows. An overnight culture was grown in brain heart infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) at the optimal growth temperature (25°C for *Y. enterocolitica* and 37°C for *S. enterica* and *L. monocytogenes*). To obtain cells in a late logarithmic growth phase, 100 μl of the overnight culture was inoculated into 10 ml of BHI broth and grown for additional 6 to 8 h at the appropriate temperature. *Campylobacter* strains were grown at 42°C for 44 to 48 h under microaerophilic conditions and used directly for spiking. All cultures were diluted in 10-fold steps (up to $10^{-7}$ or $10^{-8}$), and 1 ml of each dilution was used for spiking 25 g of food sample.

For exact quantification of the inoculum, a plate counting assay was performed. Spiked samples were enriched according to the corresponding ISO standard methods *(1–4)*. Culture media used were buffered peptone water (BPW; Merck) and Rappaport Vassiliadis (RVS) and Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broths (Oxoid Ltd., Basingstoke, UK) for *Salmonella,* half-Fraser and Fraser broths (Merck) for *L. monocytogenes*, peptone-sorbitol-bile (PSB; Sigma-Aldrich GmbH, Vienna, Austria) and irgasan-ticarcillin-chlorate (ITC; Merck) broths for *Y. enterocolitica,* and Bolton broth (Oxoid) for *Campylobacter* spp.

A multiple pathogen spiking experiment was also performed. A minced meat (pork and beef mix) matrix was spiked with different levels of mixed *Salmonella* Typhimurium DSM 554, *L. monocytogenes* SLCC 2755, and *Y. enterocolitica* NCTC 10460

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TABLE 1. *Overview of the samples analyzed in this study*

| Sample set[a] | Organism | Food matrix[b] | Preenrichment | Enrichment | Quantified spiking levels (CFU/25 g of food)[c] |
|---|---|---|---|---|---|
| 1 | *L. monocytogenes* SLCC 2755 | Cheese | Half-Fraser broth (24 h at 30°C) | Fraser broth (24 h at 37°C) | 0, 5, 45 |
| 2 | *L. monocytogenes* SLCC 2755 | Cheese | Half-Fraser broth (24 h at 30°C) | Fraser broth (24 h at 37°C) | 0, 41, 295 |
| 3 | *L. monocytogenes* NCTC 5105 | Cheese | Half-Fraser broth (24 h at 30°C) | Fraser broth (24 h at 37°C) | 0, 8, 47 |
| 4 | *L. monocytogenes* NCTC 7973 | Paté | Half-Fraser broth (24 h at 30°C) | Fraser broth (24 h at 37°C) | 0, 8, 80 |
| 5A | *Salmonella* Typhimurium DSM 554 | Chicken meat | Buffered peptone water (18 h at 37°C) | MKTTn broth (24 h at 37°C) | 0, 17, 118 |
| 5B | *Salmonella* Typhimurium DSM 554 | Chicken meat | Buffered peptone water (18 h at 37°C) | RVS broth (24 h at 42°C) | 0, 17, 118 |
| 6 | *Salmonella* Typhimurium DSM 554 | Chicken meat | Buffered peptone water (18 h at 37°C) | RVS broth (24 h at 42°C) | 0, 6, 57 |
| 7 | *Salmonella* Enteritidis DSM 9898 | Egg | Buffered peptone water (18 h at 37°C) | RVS broth (24 h at 42°C) | 0, 5, 30 |
| 8 | *C. jejuni* DSM 4688 | Chicken meat | | Bolton broth (48 h at 42°C, microaerophil) | 0, 74, 307 |
| 9 | *C. coli* DSM 4689 | Pork meat | | Bolton broth (48 h at 42°C, microaerophil) | 0, 10, 112 |
| 10 | *Y. enterocolitica* NCTC 10460 | Pork meat | | PSB broth (5 days at 25°C) | 0, 7, 74, 740 |
| 11 | *Y. enterocolitica* NCTC 10460 | Pork meat | | PSB broth (5 days at 25°C) | ND |
| 12 | *Y. enterocolitica* NCTC 10460 | Pork meat | | ITC broth (48 h at 25°C) | 0, 6, 33 |
| 13 | *Y. enterocolitica* NCTC 10460 | Pork meat | | ITC broth (48 h at 25°C) | 0, 3, 30 |
| 14 | Mixed sample | Mixed minced meat (beef + pork) | Buffered peptone water (18 h at 37°C) | Fraser broth (24 h at 37°C)<br>RVS broth (24 h at 42°C)<br>ITC broth (48 h at 25°C) | 0, 9, 64[d]<br>0, 8, 70[e]<br>0, 7, 50[f] |

[a] Sample sets 1 through 13 consisted of 9 to 12 samples (three biological replicates for each spiking level). Sample set 14 consisted of 36 samples (one set of 9 samples for preenrichment and for each enrichment).
[b] All food matrices were used raw.
[c] Quantification of the overnight culture that was used for spiking (standard plate count). ND, not done.
[d] Spiking levels for *L. monocytogenes* SLCC 2755.
[e] Spiking levels for *Salmonella* Typhimurium DSM 554.
[f] Spiking levels for *Y. enterocolitica* NCTC 10460.

cultures. Samples were preenriched in BPW for 18 h at 30°C and then selectively enriched in RVS, Fraser, and ITC broths for *S. enterica, L. monocytogenes,* and *Y. enterocolitica,* respectively. The selective enrichment broths were incubated following the corresponding ISO protocols.

After enrichment, all samples were analyzed on selective agar plates according to the standard ISO protocols. In parallel, microarray analysis was performed with each enrichment broth using a previously published microarray for the detection of food- and waterborne pathogens and indicator organisms, which enables the detection of 24 bacterial pathogens and indicator organisms, including the four target organisms and the most likely commensal flora *(8)*. Microorganisms were collected from 2 ml of enrichment supernatant by centrifugation, and total genomic DNA was extracted with the Nucleospin tissue kit (Clontech-Takara Bio Europe, Saint-Germain-en-Laye, France) according to the manufacturer's instructions and eluted in 100 μl of buffer BE. Subsequently, 5 μl of genomic DNA was used as a template for *gyrB* PCR amplification. PCR amplification, labeling, and hybridization were performed as described previously *(8)*.

An internal control was used for microarray data normalization. Obtained signals were normalized to the signal of the control oligonucleotide and expressed as a percentage (100% equals the signal of the control oligonucleotide). Normalized signals with values greater than 10% were considered unambiguously positive, and signals between 5 and 10% were considered borderline. An overview of all tested samples is given in Table 1.

## RESULTS AND DISCUSSION

Four independent spiking experiments were performed with *L. monocytogenes* (sample sets 1 through 4; Table 1). Sampling was performed after Fraser enrichment, and all samples tested positive for *L. monocyto-*

TABLE 2. *Results of the parallel spiking of mixed minced meat with* Salmonella *Typhimurium DSM 554,* L. monocytogenes *SLCC 2795, and* Y. enterocolitica *NCTC 10460*[a]

| spike level cfu / 25 g food[b] | enrichment[c] | Msi_294 | Msi_294 | Prt_2152,txt | Prt_1505,txt | Sal_1451,txt | Sal_1457,txt | Sal_1950,txt | Aer_1195,txt | Aer_1374,txt | Aer_1585,txt | Aer_1984,txt | Yer_1740,txt | Yen_1512,txt | Yen_1425,txt | Yer_1407,txt | Yer_1704,txt | Citro_1145,txt | Citro_1150,txt | Lmono_2172 | Lmono_2247 | Lmono_2343 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | BPW | 109 | 91 | 4 | 0 | 0 | 0 | 0 | 41 | 78 | 118 | 19 | 56 | 29 | 4 | 29 | 18 | 11 | 10 | 0 | 0 | 0 |
| 0 | BPW | 81 | 119 | 4 | 0 | 0 | 1 | 0 | 65 | 150 | 135 | 81 | 60 | 22 | 1 | 65 | 8 | 2 | 0 | 0 | 0 | 0 |
| 0 | BPW | 83 | 117 | 16 | 0 | 0 | 1 | 0 | 192 | 162 | 182 | 54 | 71 | 5 | 1 | 6 | 2 | 6 | 0 | 0 | 0 | 0 |
| 1 - 10 | BPW | 93 | 107 | 7 | 0 | 85 | 201 | 105 | 138 | 153 | 174 | 77 | 72 | 10 | 2 | 7 | 7 | 6 | 0 | 0 | 0 | 0 |
| 1 - 10 | BPW | 77 | 123 | 5 | 0 | 33 | 126 | 72 | 60 | 90 | 125 | 42 | 79 | 6 | 1 | 4 | 6 | 12 | 1 | 0 | 0 | 0 |
| 1 - 10 | BPW | 104 | 96 | 5 | 0 | 6 | 40 | 13 | 16 | 32 | 41 | 10 | 61 | 4 | 0 | 5 | 3 | 2 | 0 | 0 | 0 | 0 |
| 10 - 100 | BPW | 63 | 137 | 6 | 0 | 56 | 401 | 152 | 78 | 56 | 75 | 16 | 77 | 26 | 2 | 23 | 16 | 4 | 1 | 0 | 0 | 0 |
| 10 - 100 | BPW | 81 | 119 | 4 | 0 | 26 | 136 | 55 | 28 | 73 | 48 | 13 | 66 | 5 | 1 | 5 | 6 | 3 | 0 | 0 | 0 | 0 |
| 10 - 100 | BPW | 114 | 86 | 2 | 0 | 32 | 266 | 75 | 19 | 51 | 39 | 11 | 32 | 26 | 1 | 21 | 11 | 3 | 0 | 0 | 0 | 0 |
| 0 | RVS | 94 | 106 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | RVS | 95 | 105 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | RVS | 90 | 110 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 - 10 | RVS | 99 | 101 | 1 | 1 | 47 | 381 | 140 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| 1 - 10 | RVS | 84 | 116 | 0 | 1 | 40 | 311 | 95 | 0 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| 1 - 10 | RVS | 97 | 103 | 0 | 0 | 45 | 365 | 123 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | RVS | 91 | 109 | 1 | 1 | 68 | 557 | 149 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | RVS | 49 | 151 | 1 | 0 | 47 | 357 | 108 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 1 | 10 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | RVS | 100 | 100 | 0 | 0 | 26 | 232 | 70 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 |
| 0 | ITC | 86 | 114 | 11 | 1 | 0 | 1 | 0 | 122 | 122 | 199 | 61 | 339 | 40 | 3 | 31 | 197 | 28 | 61 | 0 | 0 | 0 |
| 0 | ITC | 77 | 123 | 4 | 0 | 0 | 0 | 0 | 63 | 121 | 140 | 46 | 117 | 3 | 1 | 63 | 49 | 0 | 0 | 0 | 0 | 0 |
| 0 | ITC | 77 | 123 | 2 | 0 | 0 | 0 | 0 | 16 | 25 | 25 | 10 | 38 | 36 | 2 | 17 | 14 | 0 | 0 | 0 | 0 | 0 |
| 1 - 10 | ITC | 57 | 143 | 3 | 0 | 0 | 1 | 0 | 75 | 146 | 177 | 57 | 148 | 18 | 2 | 22 | 29 | 0 | 0 | 0 | 0 | 0 |
| 1 - 10 | ITC | 82 | 118 | 4 | 0 | 0 | 1 | 0 | 67 | 144 | 256 | 64 | 154 | 1 | 0 | 1 | 6 | 1 | 2 | 0 | 0 | 0 |
| 1 - 10 | ITC | 99 | 101 | 1 | 0 | 0 | 0 | 0 | 24 | 43 | 50 | 22 | 26 | 5 | 1 | 4 | 6 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | ITC | 91 | 109 | 0 | 0 | 0 | 0 | 0 | 18 | 28 | 24 | 10 | 41 | 2 | 0 | 1 | 3 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | ITC | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 10 | 16 | 24 | 6 | 34 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | ITC | 106 | 94 | 21 | 1 | 0 | 0 | 1 | 15 | 21 | 24 | 11 | 52 | 2 | 0 | 1 | 8 | 0 | 0 | 1 | 1 | 0 |
| 0 | Fraser | 98 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | Fraser | 92 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | Fraser | 101 | 99 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 - 10 | Fraser | 99 | 101 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 124 | 178 | 142 |
| 1 - 10 | Fraser | 96 | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 58 | 44 |
| 1 - 10 | Fraser | 95 | 105 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 59 | 99 | 73 |
| 10 - 100 | Fraser | 95 | 105 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | Fraser | 92 | 108 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 - 100 | Fraser | 94 | 106 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 17 | 29 | 23 |
| | | | | *Proteus* spp. | | *Salmonella* spp. | | | *Aeromonas* spp. | | | | *Yersinia* spp. + *Y. enterocolitica* | | | | | *Citrobacter* spp. | | *L. monocytogenes* | | |

[a] Microarray hybridization results were normalized to the signal obtained from the internal control oligonucleotide (Msi_294) and expressed as percentages, where 100% is the signal for the control. Only positive signals are reported (three biological replicates per sample). Normalized signals with values above 10% were considered unambiguously positive (black boxes), and values between 5 and 10% were considered borderline (gray boxes).

[b] Target spiking levels. For actual values, see Table 1.

[c] BPW, buffered peptone water; RVS, Rappaport Vassiliadis broth; ITC, irgasan-ticarcillin-chlorate broth.

*genes,* even at the lowest spiking level of 5 CFU/25 g of food (sample set 1). Spiked cheese samples were free of any other organisms targeted by the microarray, whereas the paté sample was naturally contaminated with *L. monocytogenes*. However, this contamination could not be detected on selective chromogenic *Listeria* agar (Oxoid) and Palcam agar (BiokarDiagnostics/Solabia, Pantin, France) plates by the reference method after half-Fraser and Fraser enrichment.

For *S. enterica,* two selective media were tested: RVS and MKTTn broths. The specificity of MKTTn broth was questionable; we were not able to detect spiked *Salmonella* strains with the microarray (even at the spiking level of 118 CFU/25 g of food; see sample set 5A, Table 1). Instead, we detected *Proteus* spp., *Escherichia coli,* and *Pseudomonas aeruginosa*. The loop inoculation of MKTTn broth culture onto xylose lysine desoxycholate (XLD) agar indicated that the background flora (*Proteus* and *Citrobacter* coliforms and *Pseudomonas* spp.) growth was equal to that of the target organism, *S. enterica*. In parallel, samples were enriched in RVS broth, in which *S. enterica* was detectable at 17 CFU/25 g of food (sample set 5B, Table 1). RVS broth was more efficient for suppressing the growth of background flora, and only *E. coli* survived. Additional experiments confirmed that after RVS enrichment *S. enterica* could be readily detected by the microarray analysis even at the level of 5 CFU/25 g of food (sample set 7, Table 1). Growth of other microorganisms (*Proteus* spp., *E. coli,* and *P. aeruginosa*) was obtained in the chicken meat matrix, but no other targeted microorganisms could be detected in egg.

Pork meat samples spiked with *Y. enterocolitica* were initially enriched in PSB broth for 5 days at 25°C without agitation. During our experiments, PSB broth production was discontinued by the manufacturer, and an alternative medium had to be found. Therefore, final enrichments were performed in ITC broth for 48 h at 25°C. In total, four sets of samples were obtained (sample sets 10 through 13, Table 1), two for each of the tested media. Although PSB broth is a standard culture medium recommended by ISO *(3),* it appeared unsuitable for the specific enrichment of *Y. enterocolitica* in this study. Microarray-based analysis completely failed to detect *Y. enterocolitica* even at the highest spiking level of 740 CFU/25 g of food (sample set 10, Table 1). However, microarray results indicated the presence of a range of other organisms: *Proteus* spp., *E. coli, Aeromonas hydrophila,* and *Citrobacter* spp. The microbiological confirmation of atypical pink colonies on *Yersinia*-selective cefsulodin-irgasan-novobiocin (CIN) agar with the API 20E test (bioMérieux, Marcy l'Etoile, France) revealed that especially *Aeromonas* spp. had overgrown *Y. enterocolitica*. In addition, massive growth of background flora, which was composed predominately of *Pseudomonas* spp., *Proteus* spp., and coliform bacteria, was observed. ITC broth also allowed the coenrichment of *Proteus* spp., *A. hydrophila,* and *Citrobacter* spp., but detection of *Y. enterocolitica* at 30 CFU/25 g of food was still possible (sample set 13, Table 1).

Chicken and pork meat were used as food matrices for spiking experiments with *Campylobacter* spp. Bolton broth under microaerophilic conditions with a higher incubation temperature (42°C) provided highly specific conditions for the enrichment of *Campylobacter* spp. Microarray analysis was performed on two sample sets and revealed that *Campylobacter* spp. could be readily detected at 10 CFU/25 g of sample (sample set 9, Table 1).

The final experiment involved parallel spiking of mixed minced meat with *Salmonella* Typhimurium, *L. monocytogenes,* and *Y. enterocolitica*. Microarray analysis performed

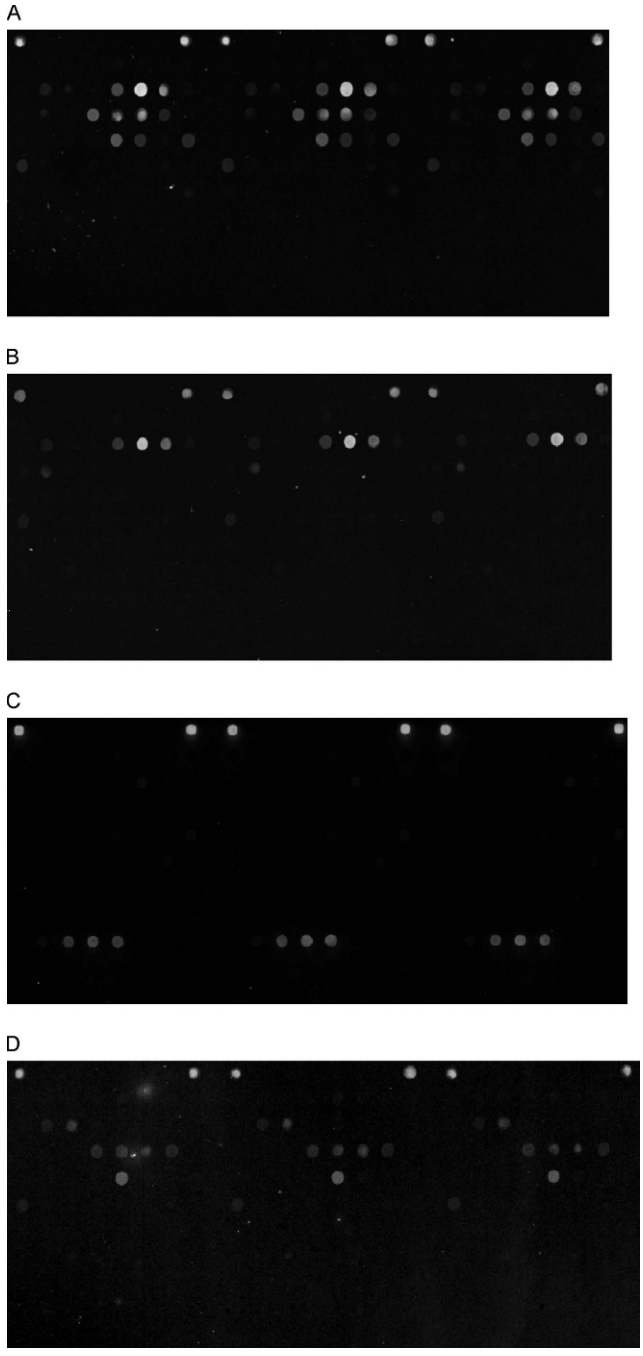


FIGURE 1. *Results of the parallel spiking of mixed minced meat with* Salmonella *Typhimurium DSM 554,* L. monocytogenes *SLCC 2795, and* Y. enterocolitica *NCTC 10460. Microarray images show hybridization patterns of one sample (spiking level, 10 to 100 CFU/25 g of meat) after enrichment in various culture media: (A) buffered peptone water, (B) Rappaport Vassiliadis broth, (C) Fraser broth, and (D) irgasan-ticarcillin-chlorate broth.*

after initial nonspecific preenrichment in BPW confirmed the complexity of the selected matrix. In the nonspiked controls, *Proteus* spp., *A. hydrophila, Yersinia* spp., and *Citrobacter* spp. were detected. The negative control sample tested negative on conventional selective media (XLD, CIN, and Palcam agars). The results of the ensuing specific enrichment demonstrated the effect that the choice of the enrichment medium has on the outcome of the analysis (Table 2 and Fig. 1).

In conclusion, of the two enrichment and cultivation procedures used for *S. enterica,* only RVS broth can be recommended; it allowed detection of spiked cells at low levels and the growth of background microflora was mostly inhibited. For *L. monocytogenes,* enrichment in Fraser broth was suitable and suppressed growth of other competing microorganisms. Unexpected results were obtained for *Y. enterocolitica.* Although *Yersinia* spp. signals were detected in the nonspiked samples, the signal was suppressed below detectable levels in the selective PSB broth culture. Both cultivation and microarray-based detection results were negative for *Yersinia* spp. (even for the samples into which *Y. enterocolitica* was spiked). *Proteus* spp. and *Aeromonas* spp. were always detected. Our results clearly showed that the application of various (recommended) enrichment procedures can lead to very different results, indicating the need to assess the specificity of cultivation procedures in more detail. Only a few of the mentioned selective media, e.g., Bolton broth, selectively and specifically supported the growth of the target organism. MKTTn, PSB, and ITC broths did not facilitate the growth of the spiked strains *Salmonella* Typhimurium DSM 554 and *Y. enterocolitica* NCTC 10460. These target organisms were outcompeted by the gram-negative background flora. Singer and coworkers *(10)* found similar results in their investigation and pointed out that the bias introduced by cultivation could dramatically influence the outcome of *S. enterica* surveillance systems and hinder traceback investigations during *S. enterica* infection outbreaks. Similarly, Arnold and coworkers *(6)* had problems isolating and identifying *Y. enterocolitica* in minced meat. Based on the phenotype of the colonies, it was possible to detect *Yersinia*-like growth on CIN agar in only 26% of the samples. Only in one sample (0.5%), a *Y. enterocolitica* strain was detected by all investigated methods.

In this pilot study, we clearly showed the questionable specificity of routinely used enrichment media and standard detection methods. However, these issues can be simply and reliably addressed and evaluated using microarray technology. Because cultivation-based ISO methods still represent the ''gold standard'' for food safety surveillance, performance issues must be taken into more detailed consideration.

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