Comparison of Enrichment Conditions for Rapid Detection of Low Numbers of Sublethally Injured Escherichia coli O157 in Food

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ABSTRACT

A comparative study of lag phases and growth rates of healthy, stressed, and sublethally injured Escherichia coli O157 cells in 10 enrichment broths was performed. The evaluation of enrichment protocols was validated by different end point detection methods (two PCR and two combined capture-plate methods). Tryptic soy broth b [TSB (b)] provided the fastest growth (μ \(_{\text{max}} = 1.00 \pm 0.06 \text{ h}^{-1}\) but failed to recover oxidative-stressed E. coli O157. TSB (a), TSB–yeast extract medium, TSB supplemented with 8 mg/liter novobiocin plus 16 mg/liter vancomycin (TSB+), buffered peptone water (BPW), and BPW supplemented with 8 mg/liter vancomycin (BPW+V) enabled resuscitation of E. coli O157 cells independent from precurtional conditions. Modified TSB plus 10 mg/liter novobiocin (mTSB+N), EC medium, EC reduced bile salts medium (ECred), TSB, and TSB supplemented with 8 mg/liter novobiocin plus 16 mg/liter vancomycin plus 2 mg/liter rifampin plus 1 mg/liter K-Telluriet plus 1.5 g/liter bile salts no. 3 (TSB++) all failed to recover E. coli O157 cells for at least one type of stress. The use of TSB (a), TSB+, BPW, and BPW+V was compared with that of mTSB+N (International Organization for Standardization reference broth) for reliable detection of low numbers of healthy, stressed, and sublethally injured E. coli O157 (approximately 10 CFU/10 g) from foods (sprouted seeds, fermented sausage, raw milk, and raw ground beef). When low numbers of healthy cells were inoculated, BPW, BPW+V, TSB, TSB+, and mTSB+N enabled growth until detectable numbers within 6 h of enrichment at 41.5°C. Results showed that mTSB+N failed to recover to detectable numbers E. coli O157 cells sublethally injured by freeze and food stresses, in contrast to what was obtained with BPW and BPW+V. This study highlights that using mTSB+N for recovery of E. coli O157 from foods may yield false-negative results.

Consumption of undercooked ground beef, fermented sausage, raw milk, dairy products, fresh produce, sprouted seeds, or apple cider has been associated with foodborne outbreaks of illness caused by Escherichia coli O157 (26, 27). A low infectious dose (1 to 100 cells) (28), the ability to survive in various foods (6, 7, 24), and the severity of illness (32) make E. coli O157 one of the most notorious foodborne pathogens (22, 23). As most of the implicated foods are highly perishable, rapid and reliable methodology for E. coli O157 detection is needed. Thus, the detection procedure must provide minimal false-positive results to ensure that the product is not needlessly discarded. Meanwhile, false-negative results cannot be tolerated because they would allow contaminated products to be released.

Due to the low numbers of E. coli O157 and their stressed or injured state, resuscitation and enrichment are required prior to selective plating (5, 19). It is therefore of utmost importance to incorporate an appropriate sample preparation protocol including an enrichment step in order to avoid false-negative results (3). The first step of a sample preparation protocol is the selection of an appropriate enrichment broth capable of resuscitating the sublethally injured target in the presence of nontarget accompanying flora. Nevertheless, suppression of nontarget microorganisms by selective supplements in enrichment broth should not compromise the fast recovery of injured and physiologically deficient target microorganisms (18). The selectivity needed for detection can be added in the actual detection step, which follows the enrichment step. Cells can be selectively taken from a suspension, for example, by antibodies attached to a solid phase, or they can be selectively detected by PCR (9, 29).

In this study the resuscitation and enrichment efficiencies of 10 different enrichment broths were evaluated using lag phase (λ) and maximum specific growth rate (μ \(_{\text{max}}\)) values obtained with each of these broths. The experimental setup included both healthy cells and cells stressed by freeze, food, and oxidative stresses (19). Next, selected enrichment broths were combined with either real-time PCR (RT-PCR) or two selected magnetic separation techniques, coupled with subsequent plating on selective agar media, to assess their capacity to provide fast and specific detection of low numbers of E. coli O157 (approximately 10 CFU/10 g).

MATERIALS AND METHODS

Bacterial strains and culture conditions. A reference stock of E. coli O157 LFMFP 463 was kept at −70°C in tryptone soy broth (TSB; Oxoid, Basingstoke, England) supplemented with 0.6% (wt/wt) yeast extract (YE; Oxoid), with 15% (vol/vol)
glycerol (Prolabo, Heverlee, Belgium). A stock culture was kept at 4°C on tryptose soy agar (TSA; Oxoid) slants supplemented with 0.6% YE. The stock culture was refreshed monthly. Working cultures were prepared by suspending a loopful (1 μl) in 10 ml of fresh TSBYE and then were incubated for 24 h at 37°C.

**Broths used.** Ten broths were selected: TSB brand a [TSB (a)], TSB (b), TSBYE, mTSB+N (modified TSB [Oxoid] plus 10 mg/liter novobiocin [Oxoid]), EC (Oxoid), ECred (EC reduced bile salts [Oxoid]), TSB+ (TSB [Oxoid] plus 8 mg/liter novobiocin [Sigma, Bornem, Belgium] plus 16 mg/liter vancomycin [Sigma]), TSB++ (TSB [Oxoid] plus 8 mg/liter novobiocin plus 16 mg/liter vancomycin plus 2 mg/liter rifampicin [Sigma] plus 1 mg/liter K-Telluriet [Sigma-Aldrich, Bornem, Belgium] plus 1.5 g/liter bile salts no. 3 [Oxoid]), buffered peptone water (BPW; BioMérieux, Marcy l’Etoile, France), and BPW+V (BPW [BioMérieux] plus 8 mg/liter vancomycin).

**Determination of μ\text{max} and λ\text{control} in various broths.** Fresh cultures (24 h) were 10-fold diluted, and ca. 3.5 log CFU was inoculated (300-μl total volume) in the first well of 96-well microtiter plates (Greiner bio-one, Wemmel, Belgium). Twofold dilutions were made along rows of the microtiter plate, and triplicate experiments were performed. The initial inoculum level was confirmed by plating on TSAYE and incubation for 24 h at 37°C, providing a value referred to as log CFU/ml\text{blank}. Growth at 37°C was determined by optical density (OD) measurements taken at 10-min intervals by using a VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 380 nm. For each OD reading the OD\text{blank} value (of noninoculated broth) was subtracted from the OD reading, and the average of three replicates was calculated to obtain ΔOD growth curves. For each broth, in a prior experiment (OD measurement of a twofold serial dilution of a working culture with a defined amount in CFU per milliliter), the minimum number of cells needed to record a change in ΔOD (ΔOD > 0) had been established, resulting in a value referred to as log CFU/ml\text{detection}. The difference in the times needed for two successive (twofold) dilutions to reach a fixed ΔOD was used as a measure of the generation time. The data obtained from wells inoculated with approximately 3,000 and 1,500 healthy cells were used to determine the generation time. Low inoculum levels (<1,000 cells) were not included to eliminate the risk of encountering the variability in the length of the lag phase of individual cells as described elsewhere (8). The maximum specific growth rate (μ\text{max}) was calculated as shown in equation 1. Triplicate experiments were performed. A detailed protocol has been previously published (18).

\[
\mu_{\text{max}} = \frac{\log (2)}{\text{Generation time}}
\]  

(1)

The variation in μ\text{max} levels was tested by one-way analysis of variance (SPSS, Inc., Chicago IL). The significance of observed differences in μ\text{max} among different broths was assessed by Tukey’s honestly significant difference test (α = 0.05) in post hoc analyses. The results of the analysis of variance are indicated in Table 1 with Tukey-based homogeneous subsets (groups A, B, C, and D; significant difference for α values of 0.05).

**λ\text{control} was determined as shown in equation 2. Time to detection (TTD\text{control}) of the cells was obtained using the linear regression (R\textsuperscript{2} > 0.98) and the point of ΔOD set at 0.

\[
\lambda_{\text{control}} = \frac{(\log \text{CFU/ml}_{\text{initial}} - \log \text{CFU/ml}_{\text{detection}})}{\mu_{\text{max}} \times \text{TTD}\text{control}/\mu_{\text{max}}}
\]

(2)

**Table 1. Average μ\text{max} and standard deviation of E. coli O157 LFMFP 463 in 10 different broths at 37°C**

<table>
<thead>
<tr>
<th>Broth</th>
<th>μ\text{max} (h\textsuperscript{-1}) \textsuperscript{a}</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSB (a)</td>
<td>0.94 AB</td>
<td>0.06</td>
</tr>
<tr>
<td>TSB (b)</td>
<td>1.00 A</td>
<td>0.06</td>
</tr>
<tr>
<td>TSBYE</td>
<td>0.91 ABC</td>
<td>0.09</td>
</tr>
<tr>
<td>EC</td>
<td>0.28 D</td>
<td>0.06</td>
</tr>
<tr>
<td>EC red</td>
<td>0.75 c</td>
<td>0.05</td>
</tr>
<tr>
<td>mTSB+N</td>
<td>0.82 BC</td>
<td>0.06</td>
</tr>
<tr>
<td>TSB+</td>
<td>0.88 ABC</td>
<td>0.03</td>
</tr>
<tr>
<td>TSB++</td>
<td>0.25 D</td>
<td>0.04</td>
</tr>
<tr>
<td>BPW</td>
<td>0.83 ABC</td>
<td>0.06</td>
</tr>
<tr>
<td>BPW+V</td>
<td>0.78 BC</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Homogeneous subsets are based on Tukey’s honestly significant difference test (groups A, B, C, and D) with significant difference set at α levels of 0.05.

**Determination of λ\text{stress} in various broths.** A standardized procedure to evoke significant percentage of sublethally injured E. coli O157 cells was determined in previous work (19). Briefly, three stress models were included (i) freeze stress (10 days at −21°C), (ii) food stress (pH 4.9, 13.5% NaCl, 10 days at 4°C), and (iii) oxidative stress (750 μM H\textsubscript{2}O\textsubscript{2}, 1 h at 37°C). After stress treatment, ca. 3.5 log of E. coli O157 culture was inoculated in the wells of the first column of the microtiter plate as described above. The same suspensions of stressed cells were used for the different enrichment broths to eliminate minor variation between stressed cultures. λ\text{stress} is calculated as shown in equation 3. The growth rate (μ\text{max}), on the other hand, is not affected by the sublethal injury (18).

\[
\lambda_{\text{stress}} = \frac{(\log \text{CFU/ml}_{\text{initial}} - \log \text{CFU/ml}_{\text{detection}})}{\mu_{\text{max}}} + \frac{\mu_{\text{max}} \times \text{TTD}_{\text{stress}}}{\mu_{\text{max}}}
\]

(3)

**Microbiological analysis of food samples.** Ten grams per sample was aseptically transferred to a sterile filter stomacher bag. One 10-fold dilution was made in peptone physiological saline (8.5 g/liter NaCl and 1 g/liter neutralized bacteriological peptone [Oxoid]), and the sample was homogenized for 60 s by means of a stomacher Seward Laboratory blender 400 (UAC House, London, England). Subsequently, a decimal dilution series in peptone physiological saline was made, and enumeration on plate count agar (Oxoid) was performed to determine the total aerobic plate count. After an incubation period of 3 days at 30°C, CFU were counted. Similarly, coliform count was determined on violet red bile lactose agar (Bio-Rad) incubated for 24 h at 37°C. E. coli organisms were enumerated on COLI ID (BioMérieux) with incubation of 24 h at 44°C. All enumerations were done by pour plate technique.

**Preparation and enrichment of artificially contaminated samples.** Ten grams of each food type (fermented sausage, raw minced beef, raw soy sprouts, and raw milk) was artificially contaminated with approximately 10 E. coli O157 LFMFP 463 cells by adding 100 μl of appropriate dilutions. To mimic real case scenarios of naturally contaminated samples, not only healthy cells of E. coli O157 LFMFP 463 but also stressed and sublethally injured E. coli O157 cells were inoculated. The fermented sausage was inoculated with food-stressed cells, the raw minced beef was inoculated with freeze-stressed cells, and the raw soy sprouts were inoculated with oxidative-stressed cells. For the raw milk samples,
healthy cells as well as all three types of stressed and sublethally injured cells were inoculated. Selected enrichment broths (BPW/ BPW+V, mTSB+N, TSB+, and TSB) were compared for their capacity to recover cells in combination with various E. coli O157 detection strategies. BPW was used for raw minced beef, and BPW+V was used for the other food matrices.

Detection was achieved by two PCR-based methods in addition to two magnetic separation base methods, as detailed in the following section.

**E. coli O157 phage protein–based capture kit.** The protocol for the E. coli O157 phage protein–based capture kit (Profos, Regensburg, Germany) was performed as described by the manufacturer. Briefly, 1 ml of the enriched sample and 50 μl of phage protein–coated magnetic beads were incubated at room temperature in an overhead rotor for 20 min. A magnetic field was applied for 5 min, and the supernatant was carefully aspirated and discarded. Samples were washed three times with phosphate-buffered saline–Tween 20 (PBST buffer) (Sigma-Aldrich). The supernatant was discarded, and the particle-bacteria complex was resuspended in 100 μl of PBST buffer before 50 μl of the mixture was plated onto sorbitol MacConkey agar supplemented with cefixime tellurite selective supplement (CT-SMAC; Oxoid) and 50 μl on chromogenic O157 ID agar (BioMérieux). Colony counts were obtained after 24 h of incubation at 37°C.

**E. coli O157 anti-O157 Dynabeads capture.** The protocol for the E. coli O157 anti-O157 Dynabeads capture (Dynal, Oslo, Norway) was performed as described by the manufacturer. Briefly, 1 ml of the enriched sample and 20 μl of protein coated magnetic beads were incubated at room temperature in an overhead rotor for 10 min. A magnetic field was applied for 3 min, and the supernatant was carefully aspirated and discarded. Samples were washed three times with phosphate-buffered saline (PBST buffer) (Sigma-Aldrich). The supernatant was discarded, and the particle-bacteria complex was resuspended in 100 μl of PBST buffer before 50 μl of the mixture was plated onto sorbitol MacConkey agar supplemented with cefixime tellurite selective supplement (CT-SMAC; Oxoid) and 50 μl on chromogenic O157 ID agar (BioMérieux). Colony counts were obtained after 24 h of incubation at 37°C.

**TaqMan E. coli O157:H7 PCR kit.** The protocol for TaqMan E. coli O157:H7 PCR kit (Applied Biosystems, Lennik, Belgium) was performed as described by the manufacturer. Briefly, 1 ml of the enriched samples was centrifuged (centrifuge 5415 C, Eppendorf, Hamburg, Germany) (room temperature, 10 min, 1,000 X g), and the supernatant was discarded. DNA was extracted by adding 100 μl of lysis reagent (PrepMan Ultra, Applied Biosystems) to the pellet and heating for 10 min at 95 to 100°C. DNA extracts were diluted 1/10 by adding RNase-free water (Qiagen, Hilden, Germany), and 12 μl of the obtained diluted DNA solutions was used for the amplification reaction (18 μl of PCR mixture). Amplification consisted of 45 cycles with 15 s of denaturation at 95°C and 1 min of annealing at 60°C. Internal controls were included in each PCR.

**RESULTS**

**Determination of μmax.** Table 1 shows μmax values of E. coli O157 as a function of the enrichment broths used. Significantly higher μmax values for E. coli O157 in TSB (brads a and b), TSBYE, TSB+, and BPW were obtained, compared with a subset of intermediate μmax values obtained in mTSB+N and in BPW+V and with the subset of the lowest μmax obtained in EC, ECred, and TSB++. EC, ECred, and TSB++ can be considered to be less-growth–supporting broths.

**Determination of λcontrol and λstress.** Figure 1 shows that EC, mTSB+N, and TSB++ were not capable of resuscitating the freeze-stressed cells within 24 h. Moreover, EC and ECred did not support recovery of oxidative-stressed cells within the duration of the experiment, nor did TSB (b). In addition, mTSB+N and TSB++ also inhibited growth after a food stress.
Detection of *E. coli* O157 in artificially contaminat-
ed food samples. Details of the results of the various
detection strategies are presented in Table 2. An increase in
CFU by capture combined with plating or a decrease in
threshold cycle ($C_T$) values by RT-PCR detection can be
noticed after prolonged enrichment time. In several cases a
specific combination of enrichment broth and detection
strategy failed to detect inoculated injured cells. The
percentages of sublethally injured cells due to freeze, food,
and oxidative stress were determined to be 99.5, 99.9, and
76.2% (significant percentage of sublethal injury for the
freeze and food stress (19)), respectively. For the oxidative-
stressed cells, a prolongation in detection time was
noticeable. The type of food (and associated variation in
competitive flora) did not seem to influence the recovery of
*E. coli* O157. The total aerobic counts for soy, minced beef,
fermented sausage, and raw milk were, respectively, 7.5,
4.9, 8.2, and 3.2 log CFU/g; 7 and 3.3 log CFU/g coliforms
were found in soy and raw minced beef, respectively (for the
fermented sausage and raw milk, there were <10 CFU/g
coliforms). None of the food samples showed detectable
numbers of *E. coli* (<10 CFU/g) prior to inoculation.

In order to visualize the effects of sublethally injured
cells on the performance of enrichment (and thus detection)
strategies, the results of detection of food- and freeze-
stressed cells (Fig. 2B) and the parallel results of the healthy
cells (Fig. 2A) were analyzed in terms of sensitivity rate.
The sensitivity rate has been defined as the ratio of the
number of positive samples found over the number of
inoculated samples. A sensitivity rate of 1 means that in all
cases when food samples were inoculated the combination
of enrichment broth, enrichment time, and detection method
resulted in detection of *E. coli* O157 cells. When the
sensitivity rate was <1 but >0, at least for one food sample
a certain combination of enrichment method and detection
protocol resulted in no detection of *E. coli* O157 cells,
although the food sample was inoculated. A sensitivity of 0
revealed that the food samples were inoculated but no
detection of target cells could be achieved. Figure 2A shows
that different food samples inoculated with healthy cells of
*E. coli* O157 were all found to be positive (sensitivity rate =
1) already after 6 h of enrichment independently from
enrichment broth and detection strategy. A sensitivity rate of
1 was already obtained after 4 h of enrichment (indepen-
dently from the selected broth) if the detection strategy
involved was either *E. coli* O157 phage protein–based
Capture Kit (Profos) or TaqMan RT-PCR (Applied
Biosystems). For sublethally injured *E. coli* O157 cells
(>99% sublethal injury established), enrichment in BPW/
BPW+V enabled the maximum recovery after 6 h of
enrichment (Fig. 2) with the various detection methods,
although a sensitivity rate of 1 was not reached. Even with
6 h of incubation in BPW or BPW+V for many detection
strategies, the detection limit was indicated by only few
colonies isolated by capture on plate or high $C_T$ values.
In some cases injured cells were detected after 4 h of
enrichment with a specific detection strategy; for example,
freeze-stressed cells inoculated in raw minced meat and
food-stressed cells inoculated in fermented sausage, both of
these occurring with RT-PCR detection. However, in both
cases a $C_T$ value of 40 was obtained. mTSB+N did not
enable the resuscitation of freeze-stressed and food-stressed
sublethally injured *E. coli* O157 cells.

**DISCUSSION**

Detection of sublethally injured cells, especially
pathogens characterized with low infectious dose, such as
*E. coli* O157, is critical (31). The selectivity of the
enrichment broth is therefore a major point of attention as
selective compounds such as bile salts and antibiotics could
inhibit the recovery of injured cells (2). The first objective
of this study was to determine the best enrichment broth for
*E. coli* O157. Based on the combination of a short $\lambda$, a fast
$\mu$ max, and a resuscitation capacity that was independent from
precultural conditions, BPW, BPW+V, TSB, and TSB+ were
selected for further evaluation. In the present study,
significantly higher values for $\mu$ max of *E. coli* O157 were
obtained in the nonselective media than in the classical
selective enrichment media (Table 1). However, nonselective
broths for *E. coli* O157 cannot generally be recom-
manded for food samples with a high level of background
flora, as growth of such flora can outcompete target cells
(1). This is the reason why in the International Organization
for Standardization (ISO) methods for *E. coli* O157 (ISO
16654:2001 (15)), *Listeria monocytogenes* (ISO 11290-1
(14)), and *Campylobacter* spp. (ISO 10272-1 (17)) selective
broths, mTSB+N, Demi-Fraser & Fraser, and Bolton broths,
respectively, are recommended for enrichment purposes.
The second objective of this study was to evaluate the use of
nonselective media as enrichment broths in the detection of
*E. coli* O157 in foods with indigenous microflora. For this
purpose healthy, stressed, and sublethally injured cells were
inoculated in different food samples. Different detection
methods were included in the experimental setup to account
for a complete strategy with different detection methods.
The combination of detection technologies and formats
supports the selection of an appropriate enrichment broth. In
the present study, it was evident that mTSB+N consistently
failed to allow resuscitation and enrichment of sublethally
injured *E. coli* O157 cells. Similar false negatives were
reported elsewhere (2, 12). Based on the data obtained,
preference is given to a nonselective medium such as BPW
for enrichment of *E. coli* O157 from foods. This is further
supported by comparison of $\mu$ max of *E. coli* O157 (0.94 ±
0.06 h$^{-1}$) in TSB and $\mu$ max of *L. monocytogenes* in TSB
(0.39 ± 0.05 h$^{-1}$) (18), whereby it can be noticed that the
$\mu$ max of *E. coli* O157 is higher than the $\mu$ max of *Listeria
monocytogenes*. This implies that *E. coli* O157 is a fast
grower, comparable to *Salmonella* (ISO 6579:2005) (16),
enabling the option of a nonselective medium (BPW or
brain heart infusion broth) or medium with limited
antibiotics (BPW+V) for enrichment without running the
risk of being overgrown by the competitive flora. This has
been confirmed in the present study by the experiments with
the various food types.

This study emphasizes the shift in performance of
detection strategies (and especially the choice of the prior
<table>
<thead>
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<th>Matrix</th>
<th>Physiological state</th>
<th>Time (h)</th>
<th>Level of detection obtained by:</th>
<th>C&lt;sub&gt;T&lt;/sub&gt; value obtained by:</th>
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<td>E. coli O157 Capture kit</td>
<td>Anti-O157 Dynabeads assay</td>
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<td>BPW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>mTSB+N</td>
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<td>Soy</td>
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<td>Sublethally injured cells (food)</td>
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<tr>
<td></td>
<td>Sublethally injured cells (food)</td>
<td>24</td>
<td>-</td>
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</table>

<sup>a</sup> -, no CFU detected or (for RT-PCR) no signal; +, 1 to 14 CFU per plate; ++, 15 to 150 CFU per plate; ++++, >151 CFU per plate; Inh, inhibition of PCR.

<sup>b</sup> BPW was used for raw minced beef, and BPW+V was used for soy, fermented sausage, and raw milk.
and is included as part of the ISO standard method (15). In this case the sensitivity and the specificity of the detection technique are in the first place based on the choice of the antibody early during development of a certain kit.

Overall, in the present study, the phage protein–based capture supported detection of E. coli O157 (both healthy and injured cells) faster (i.e., occasionally after only 4 h of enrichment time) than the antibody-based capture. This can be explained by the higher affinity (and thus sensitivity) of bacteriophage proteins. Phage proteins (from bacteriophages) are more specifically attached to bacteria than antibodies (20). However, the inclusion of the capture step takes its toll in the total length of the detection procedure, as it still requires a 24-h incubation of the captured cells on the selective (chromogenic) agar media. A solution to this is to use the captured cells for DNA extraction and PCR detection to obtain a faster result. However, from the present study it has been shown that concentration of cells by capture is not a necessary intermediate step for successful PCR detection. It was demonstrated that 1 ml of culture enriched for 6 h in BPW can be directly processed for DNA extraction and PCR, providing results within 8 h. This has been shown to be a rapid and reliable detection strategy for the recovery of low numbers of E. coli O157 (including injured) cells in food. Further experiments should be performed to determine statistically the best detection strategy for fast and reliable detection of E. coli O157 from different food samples after a nonselective or less selective enrichment broth is used. These further experiments should be performed with a wider range of foods and different replicates, preferably including an interlaboratory trial.

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FIGURE 2. Sensitivity rate of the detection protocol (combination enrichment broth and detection method) when different food samples were inoculated with approximately 10 healthy cells (A) or sublethally injured cells (B) (food- and freeze-stressed cells). A sensitivity rate of 1 implies that all inoculated food samples were found to be positive. Bars represent sensitivity rate of the detection protocol when food samples were incubated at 37°C for 4 h (III), 6 h (II), or 24 h (I). BPW*, BPW was used for raw minced beef, and BPW+N was used for soy, fermented sausage, and raw milk; **, no detection of E. coli O157 cells in food samples.

enrichment broth for the execution of the detection method) if healthy cells are used versus sublethally injured cells. It is therefore highly recommendable to use naturally contaminated samples or samples inoculated with injured cells when designing and evaluating detection strategies protocols (19). Because of the low prevalence of E. coli O157, finding naturally contaminated food samples is not easily achieved (4, 21). In order to compensate for the low prevalence of E. coli O157 in foods and still mimic natural contamination, the present study was based on stressed and sublethally injured inocula. The incorporation of cultures with stress exposure history differentiated this present study from those published elsewhere (9–11, 13).

The selectivity needed for the detection strategy can be added to the actual detection method (magnetic separation combined with plating, PCR, and enzyme-linked immunosorbent assay) that follows the enrichment step. Immunomagnetic separation using O157-specific-antibody–coated beads followed by bacteriological cultures has been shown to improve the detection of E. coli O157 in foods (25, 30).