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Evaluation of different buffered peptone water (BPW) based enrichment broths for detection of Gram-negative foodborne pathogens from various food matrices

H. Margot [a], M.H. Zwietering [b], H. Joosten [c], Emer O'Mahony [d], R. Stephan [a,*]

[a] Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, CH-8057 Zurich, Switzerland
[b] Laboratory of Food Microbiology, Wageningen University, 6700 AA Wageningen, The Netherlands
[c] European Chair in Food Safety Microbiology, Wageningen University, 6700 AA Wageningen, The Netherlands
[d] Quality and Safety Department, Nestlé Research Center, Vers-chez-les-Blanc, CH-1000 Lausanne, Switzerland

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ABSTRACT

This study evaluated the effects of changing the composition of the pre-enrichment medium buffered peptone water (BPW) on the growth of stressed and unstressed Gram-negative foodborne pathogens in a one-broth enrichment strategy. BPW supplemented with an available iron source and sodium pyruvate, along with low levels of 8-hydroxyquinoline and sodium deoxycholate (BPW-S) improved the recovery of desiccated *Cronobacter* spp. from powdered infant formula. Growth of *Salmonella* and STEC was comparable in all BPW variants tested for different food matrices. In products with high levels of Gram-negative background flora (e.g. sprouts), the target organisms could not be reliably detected by PCR in any of the BPW variants tested unless the initial level exceeded 10^3 cfu/10 g of sprouts.
Based on these results we suggest BPW-S for a one-broth enrichment strategy of stressed Gram-negative foodborne pathogens from dry products. However, a one-broth enrichment strategy based on BPW variants tested in this evaluation is not recommended for produce with a high level of Gram-negative background flora due to very high detection limits.

1. Introduction

Key to the reliability of all methods for the detection of Gram-negative foodborne pathogens (e.g. *Salmonella*, *Cronobacter* and Shigatoxin-producing *Escherichia coli*) is the capability to recover low numbers of stressed cells from any kind of food matrix to a detectable level. These methods usually consist of three consecutive steps. The pre-enrichment step, that is common to most current detection methodologies (cultural, molecular and immunological), aims at the resuscitation/recovery of cells in a non-selective medium such as buffered peptone water (BPW). The second step, a selective enrichment, should promote growth of the recovered target bacteria. The last step, the actual detection of the pathogen can be accomplished with either cultural or molecular methods.

Gram-negative foodborne pathogens are usually present in relatively low numbers, sometimes accompanied by a high number of closely related competitor organisms (Baylis et al., 2000). In addition, cells may be sub-lethally injured by food processing or intrinsic factors of the food matrix (Edel and Kampelmacher, 1973). The purpose of the pre-enrichment step is to allow stressed target microorganisms to resuscitate and grow in either a non-selective or moderately selective environment. At this stage, the target organisms can be overgrown by the background flora in the food product due to absent selectivity of the medium, which can lead to false negative results.

The selective enrichment should promote growth of the recovered target bacteria. The necessity of applying a selective enrichment will mostly depend on the characteristics of the food product, particularly on its microbial flora. In products with a high number of competing organisms, in which the target bacteria are likely to be stressed, both a non-selective and selective enrichment step will be necessary. For products with low levels of background flora, a one-broth enrichment strategy in an unselective enrichment broth can be sufficient. Since the pre-enrichment and selective enrichment step are the main time limiting factors in regard to turn around time for rapid methods (Baylis et al., 2000), a moderately selective one-broth enrichment strategy can decrease the total time until results are available.

The success of the pre-enrichment step is however not only depending on the food product but also on the quality of the medium. Preliminary work has shown that there are some inconsistencies in the performance of BPW and that the ISO standard formulation

* Corresponding author at: Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Winterthurerstr. 272, CH-8057 Zurich, Switzerland.
E-mail address: stephanr@fsafety.uzh.ch (R. Stephan).

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results in performance variations not just from manufacturer to manufacturer but also from batch to batch. A comparison of 18 different commercial brands of buffered peptone water showed differences in performance regarding buffering capacity, growth of unstressed bacteria and recovery of dry-stressed cells (unpublished data). The performance of the BPW brands depended heavily on the matrix and the organism tested. Baylis et al. (2000) compared two commercially available preparations of buffered peptone water with regard to their ability to promote recovery and growth of sub-lethally injured *Salmonella enterica*. There was a significantly higher recovery rate stressed of cells with one brand of buffered peptone water however, the performance of BPW in food was mostly dependent on the food type with the type and level of background flora present being the most important influential factors. Strain variability also exerted an influence on the recovery of stressed cells. Different studies have investigated an improvement of enrichment media by supplementation and by modification of the conditions during enrichment such as temperature, pH, time etc. (Andrews, 1986). Gray et al. (2006) investigated the effect of variation between classes of casein, gelatine and peptones from different sources. They concluded that the variation in peptone has a substantial influence when looking at growth and enumeration of bacteria. For STEC, pre-enrichment methods were developed based on meat industry requirements that are, however, inadequate for the recovery of STEC from vegetables, flour and other low a_w matrices (Sata et al., 2003). Weber et al. (2009) showed that the supplementation of growth factors such as additional iron and sodium pyruvate, along with low levels of inhibiting agents primarily against Gram-positive background flora (8-hydroxyquinoline and sodium deoxycholate), enhances the recovery rate of stressed cells.

Products containing starter cultures and probiotic cultures present a special challenge to pathogen detection. Strong acidification of the enrichment caused by lactic acid producing bacteria can lead to inactivation of target bacteria and subsequently false-negative results. Previous experimental work has shown that increased concentrations of buffer phosphates in BPW improved detection of pathogens in powders containing probiotics (unpublished data). For this reason, BPW with 3× and 6× increase in buffer phosphate concentration were included in the study. So far, there are only few studies evaluating the use of BPW with supplements in a one-broth enrichment. In particular, data obtained from experiments in the food matrix are scarce.

The aim of the current study was to compare different modifications of BPW with regard to their ability to promote growth of unstressed and stressed Gram-negative foodborne pathogens in different food matrices.

2. Materials and methods

2.1. Bacterial strains

Different *S. enterica*, *Cronobacter* spp. and Shigatoxin-producing *Escherichia coli* (STEC) strains were chosen as representatives for Gram-negative food borne pathogens (Table 1). Working cultures were made from frozen (−80 °C) BHI (Oxoid CM1135, Basingstoke, United Kingdom) with 20% glycerol stocks and maintained on blood agar plates (Difco Columbia blood agar base, 5% sheep blood, CM0031 Oxoid) at 4 ± 1 °C. All strains were natural isolates, obtained from our in-house collection.

2.2. Supplementation of BPW

Buffered peptone water (BPW, Oxoid CM1049) was prepared according to the manufacturer's instructions. BPW-S was prepared by using BPW with the following supplements added before autoclaving: 40 μM 8-hydroxyquinoline, 0.5 g/l ammonium-iron(III) citrate, 0.1 g/l sodium deoxycholate, 0.1 g/l sodium pyruvate (all from Sigma-Aldrich, Buchs, Switzerland).

Table 1
Strains used in this study.

Strain	Species
N10 2100	*Salmonella* Agona
N10 2099	*Salmonella* Rissen
N10 2103	*Salmonella* Hadar
N10 2054	*Salmonella* Livingstone
S604	*Salmonella* Anatum
N10 1898	*Salmonella* Senftenberg
N10 1905	*Salmonella enterica* O:rough
N472 962	*Salmonella* Typhimurium, monophasic
N10 2043	*Salmonella* Virchow
N10 2148	*Salmonella* London
40	*E. coli* O103
K124	*E. coli* O91:H10
N10 630	*E. coli* O128ac:H2
K406	*E. coli* O116:H28
115903	*E. coli* O55:H7
N10 632	*E. coli* O111:H8
185004	*E. coli* O121:H19
96803	*E. coli* O26:H11
N10 635	*E. coli* O26:H11
33	*E. coli* O145
E608	*Cronobacter malonaticus*
E465	*Cronobacter dublinensis*
E265	*Cronobacter malonaticus*
E288	*Cronobacter sakazakii*
E615	*Cronobacter malonaticus*
E151	*Cronobacter sakazakii*
E269	*Cronobacter sakazakii*
E266	*Cronobacter sakazakii*
E776	*Cronobacter sakazakii*
E532	*Cronobacter malonaticus*

6 × BPW (quantities for 3 × BPW in brackets) was prepared by addition of 7.5 g/l (3 g/l) KH_2PO_4 and 17.5 g/l (7 g/l) Na_2HPO_4 (anhydrous) (Sigma-Aldrich) to BPW. 6 × BPW-S contains both additional buffer salts and supplements of BPW-S. Throughout this work, buffered peptone water from a single batch was used to avoid lot-to-lot variations.

2.3. Recovery of dry stressed cells

Each ten strains of *Cronobacter* spp., STEC and *S. enterica* were grown overnight in 9 ml BHI (Oxoid) at 37 °C. Grown cultures were centrifuged at 13,000 *g* for 10 min. The supernatant was discarded and the pellet was re-suspended in 1 ml BPW. The cell suspension with cell counts of approximately 10^9–10^{10} cfu/ml was serially tenfold diluted in BPW. 10 μl of each dilution were pipetted in eight wells of a 96-well microtiter plate using one plate per strain resulting in 10^7–10^8 cfu/well in the first row. Cell counts of the culture were determined with plate counts on tryptic soy agar (BD Diagnostic Systems, Heidelberg, Germany). After two days of storage in a desiccator containing silica gel at room temperature, one complete plate was rehydrated with 200 μl BPW, BPW-S or 3 × BPW per well and incubated 16 ± 2 h at 37 °C. The most probable number (MPN) of microorganisms that had survived the drying process and were able to grow in the enrichment broth was determined based on the number of wells in which growth was observed using the Bacteriological Analytical Manual *Online* MPN table (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm).

2.4. Application of desiccation stress

In food matrices with a low a_w, pathogens are expected to have encountered desiccation stress. To achieve a more realistic scenario, cells used for spiking were desiccation stressed before inoculation.

The STEC K124 and 33, *Cronobacter* spp. E615 and E776, *S. enterica* N10 1905 and N472 962 strains were grown separately overnight in 30 ml BHI (Oxoid) at 37 °C. After centrifugation for 12 min at 4000 rpm and the supernatant was discarded. The inside of the