Food Microbiology 66 (2017) 40–47

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Comparison of *Listeria monocytogenes* recoveries from spiked mung bean sprouts by the enrichment methods of three regulatory agencies☆

Kaitlin E. Cauchon [a], Anthony D. Hitchins [b, 1], R. Derike Smiley [a, *]

[a] U.S. Food and Drug Administration, Office of Regulatory Affairs, Arkansas Regional Laboratory, 3900 NCTR Road, Building 26, Jefferson, AR, 72079, United States
[b] U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5001 Campus Drive, College Park, MD, 20740, United States

ARTICLE INFO

Article history:
Received 30 January 2017
Received in revised form
28 March 2017
Accepted 29 March 2017
Available online 5 April 2017

Keywords:
Regulatory microbiology
Listeria
Listeria innocua
Listeria monocytogenes
Selective enrichment
Fraser broth
UVM broth
BLEB

ABSTRACT

Three selective enrichment methods, the United States Food and Drug Administration's (FDA method), the United States Department of Agriculture Food Safety Inspection Service's (USDA method), and the EN ISO 11290-1 standard method, were assessed for their suitability for recovery of *Listeria monocytogenes* from spiked mung bean sprouts. Three parameters were evaluated; the enrichment *L. monocytogenes* population from singly-spiked sprouts, the enrichment *L. monocytogenes* population from doubly-spiked (*L. monocytogenes* and *Listeria innocua*) sprouts, and the population differential resulting from the enrichment of doubly-spiked sprouts. Considerable *L. monocytogenes* inter-strain variation was observed. The mean enrichment *L. monocytogenes* populations for singly-spiked sprouts were 6.1 ± 1.2, 4.9 ± 1.2, and 6.9 ± 2.3 log CFU/mL for the FDA, USDA, and EN ISO 11290-1 methods, respectively. The mean *L. monocytogenes* populations for doubly-spiked sprouts were 4.7 ± 1.1, 5.5 ± 1.3, and 4.6 ± 1.4 log CFU/mL for the FDA, USDA, and ISO 11290−1 enrichment methods, respectively. The corresponding mean population differentials were 2.8 ± 1.1, 3.3 ± 1.3, and 3.6 ± 1.4 Δlog CFU/mL for the same three enrichment methods, respectively. The presence of *L. innocua* and resident microorganisms on the sprouts negatively impacted final levels of *L. monocytogenes* with all three enrichment methods.

Published by Elsevier Ltd.

1. Introduction

Sprouts are germinated seeds and are produced by first soaking the seed in water followed by prolonged incubation of up to one week at ambient temperature and high moisture levels; these conditions are favorable for growth of foodborne pathogens. Once harvested, the sprouts are packaged and usually refrigerated for transportation and retail sale. *Listeria monocytogenes* is a ubiquitous, pathogenic, soil borne microorganism capable of growth on sprouts during all stages of production including refrigerated storage. Because of the potential severity of the illness resulting from the consumption of this organism and because sprouts are frequently consumed raw, the United States Food and Drug Administration (FDA) maintains a “zero tolerance” level for *L. monocytogenes* contamination on sprouts.

Levels of *L. monocytogenes* on sprouts can be below threshold levels needed for direct detection and recovery so regulatory agencies rely on selective enrichment as the first step to detecting and recovering this organism. The three most popular regulatory *Listeria monocytogenes* enrichment methods are the FDA's *Listeria* selective enrichment method, the United States Department of Agriculture Food Safety and Inspection Service's *Listeria* selective enrichment method (USDA enrichment method), and the international standard method for *Listeria* detection (EN ISO 11290−1 enrichment method). Despite the use of selective agents in these enrichment methods, species-specific growth is not achieved. When more than one species of *Listeria* is present in the test sample each may respond differently to selective enrichment conditions (Petran and Swanson, 1993; Curiale and Lewus, 1994; Carvalheira et al., 2010; Cornu et al., 2002; Ganou Besse et al., 2005; Keys

☆ This work is the opinion of the authors and not that of the U.S. Food and Drug Administration. Reference to commercial technologies or brands do not imply endorsement nor does omission of similar technologies or brands imply criticism by the U.S. FDA.

* Corresponding author.
E-mail address: Ronald.Smiley@fda.hhs.gov (R.D. Smiley).

[1] Retired.

http://dx.doi.org/10.1016/j.fm.2017.03.021
0740-0020/Published by Elsevier Ltd.

et al., 2013; Dailey et al., 2015; Ganou Besse et al., 2016). The importance of this observation is that recovery of only *L. innocua* or any other non-pathogenic *Listeria* species does not preclude the presence of *L. monocytogenes*. In addition to supporting the growth of non-pathogenic species of *Listeria*, these selective enrichment broths also support growth of some strains of non-*Listeria* species (Hitchins and Tran, 1990; Tran et al., 1990; Dallas et al., 1991; Al-Zehara et al., 2011; Dailey et al., 2014; Keys et al., 2016). Growth of non-*Listeria* competitors during selective enrichment may prevent *L. monocytogenes* from reaching minimum threshold levels needed for detection (Dailey et al., 2014).

The objective of this study was to compare *Listeria* inter-species competition and to evaluate the effects of non-*Listeria* competitors during the selective enrichment of spiked mung bean sprouts using three enrichment methods favored by regulatory agencies; the FDA enrichment method, USDA enrichment method and EN ISO 11290–1 enrichment method. Mung bean sprouts were selected as the test matrix since they have naturally high microbial levels, the conditions used for sprout production are conducive to growth of foodborne pathogens, and because they are frequently consumed raw. Specific aims of this study in support of the overall objective included; determining the final populations of select strains of *L. monocytogenes* in mung bean sprout enrichments, determining the population differentials between select strains of *L. monocytogenes* and *L. innocua* in doubly-spiked mung bean sprout enrichments, and estimating the contribution of non-*Listeria* microbial competitors on the final populations of select strains of *L. monocytogenes* during selective enrichment.

2. Materials and methods

2.1. Bacterial strain selection and maintenance

The ten strains of *L. monocytogenes* used in the study were specifically selected based on their competitive performance in selective buffered *Listeria* enrichment broth (BLEB) with the competitor *Citrobacter braakii* (Keys et al., 2016). The primary selection criterion was their final enrichment population; the final strain selection included a wide range of competition sensitivities. The final populations of *L. monocytogenes*, expressed as log CFU/mL, following competitive growth with *C. braakii*, were: Lm002 = 5.4, Lm007 = 4.4, Lm031 = 4.4, Lm040 = 8.0, Lm043 = 7.0, Lm053 = 7.0, Lm056 = 5.1, Lm057 = 8.0, Lm064 = 6.0, Lm070 = 6.1 (Keys et al., 2016). The purpose of this strain selection process was to prevent the introduction of bias into the results by selecting only poorly competitive or only highly competitive *L. monocytogenes* strains.

The strains of *L. innocua* were also selected based on their final populations following selective enrichment of spiked mung bean sprouts. Ten strains of *L. innocua* were initially selected for screening based on how well they reportedly competed against *C. braakii* in BLEB enrichments; all ten strains had final populations ≥7.9 log CFU/mL (Keys et al., 2016). The ten *L. innocua* strains were screened in spiked mung bean sprouts using the FDA, USDA, and EN ISO 11290–1 enrichment methods. Strain Ln018 was selected because it achieved the highest population with the FDA enrichment method and Ln056 was selected because it had the highest population for both the USDA and EN ISO 11290–1 enrichment methods. The rationale for this selection process was to ensure that the most competitive strain of *L. innocua* was used for each specific enrichment method.

All of the *Listeria* strains used in this study were initially isolated and identified using the FDA *Listeria* species isolation method (Hitchins et al., 2016). In addition, 16S rDNA sequence analysis was used to further confirm species identity (Hellberg et al., 2013). Strain Lm002 (serotype 4b) was isolated from Mexican style soft cheese, strain Lm007 (serotype 1/2b) was isolated from butter, strain Lm031 (serotype 1/2a) was isolated from a commercially produced deli-style ham and cheese sandwich, strain Lm040 and Lm056 (both serotype 1/2a) were isolated from frozen smoked salmon, strain Lm043 (serotype 1/2a) was isolated from the environmental sampling of a deli sandwich manufacturing facility, strains Lm053 and Lm070 (both serotype 1/2a) were isolated from avocado, strain Lm057 (serotype 1/2a) was isolated from frozen blueberries, strain Lm064 (serotype 1/2b) was isolated from the environmental sampling of a soft cheese manufacturing facility. All strains were maintained cryoprotectively at −80 °C and working stock cultures were maintained in motility agar deeps at room temperature.

2.2. Sprout inoculation

Mung bean sprouts were purchased at several local retailers and consisted of four brands. Although an effort was made to ensure that each brand was evenly represented, frequently brand selection was dictated by availability and so some brands were used more frequently than others. Analytical portions (25 g) of mung bean sprouts were placed into sterile 0.9 L Mason-style blender jars. The sprouts were then doubly-spiked with one of the ten strains of *L. monocytogenes* and one of the two strains of *L. innocua*. Strain Ln018 was used for FDA method enrichments and strain Ln056 was used for USDA and EN ISO 11290-1 method enrichments. All ten *L. monocytogenes* strains were represented in all three enrichment methods. For comparison, enrichments were also performed with all 10 *L. monocytogenes* strains using singly-spiked mung bean sprouts. Individual cultures of each strain were prepared from working stocks and grown 18–24 h in non-selective BLEB at 35 °C. Serial dilutions were prepared in Butterfield's phosphate buffer (BPB) (Butterfield, 1932). The target inoculum levels were 1–5 CFU/mL which were verified by plating onto tryptic soy agar supplemented with 0.6% yeast extract (TSAye) with subsequent incubation of the plates at 35 °C for 24 h. The spiked mung bean sprouts were equilibrated for 24 h at 4 °C to simulate actual regulatory test samples that would normally be received following refrigerated shipment.

2.3. Selective enrichment of spiked mung bean sprouts

Listeria selective enrichment was performed on the spiked mung bean sprouts by the FDA (Hitchins et al., 2016), USDA (Anonymous, 2010), and EN ISO 11290–1 (Anonymous, 1996) methods. All incubations were performed statically. All enrichment methods were replicated three times for each of the ten *L. monocytogenes* strains. Difco™ brand (BD Diagnostics; Starks, MD) microbiological media were used unless otherwise stated. Selective agents, buffering salts, and other chemicals were purchased from Sigma-Aldrich Chemicals (St. Louis, MO) unless otherwise specified. Oxoid™ University of Vermont modified broth (UVM-1) (Remel, Inc.; Lenexa, KS), Oxoid™ Fraser broth, and Oxoid™ demi-Fraser broth were prepared following manufacturer's instructions.

2.4. Matrix-free enrichments

Matrix-free enrichment studies were performed as previously reported (Keys et al., 2013, 2016; Dailey et al., 2014) and in a manner similar to that described in section 2.2. Briefly, 25 mL of enrichment broth were inoculated at a target level of 1–5 CFU/mL with each test strain(s). The primary incubation for the matrix-free enrichments was performed using 50 mL polystyrene, screw-capped, conical tubes. For the FDA enrichment method, selective additives were added following the initial four hours incubation as