Food Microbiology 63 (2017) 123–128

Contents lists available at ScienceDirect

Food Microbiology

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

Relative effectiveness of selected preenrichment media for the detection of *Salmonella* from leafy green produce and herbs

Andrew P. Jacobson*, Hua Wang, Vikas S. Gill [1], Robert Duvall, Gabriela Arce [2], Stuart Chirtel, Thomas S. Hammack**

U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 5001 Campus Dr., College Park, MD 20740, USA

ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form
17 October 2016
Accepted 11 November 2016
Available online 12 November 2016

ABSTRACT

Four buffered preenrichment media (BAX® System MP Media (BAX)), Universal Preenrichment Broth (UPB), modified Buffered Peptone Water (mBPW), and Buffered Peptone Water (BPW)) were compared with lactose broth (LB) in the *Bacteriological Analytical Manual's* (BAM) *Salmonella* culture method for the analysis of 9 leafy green produce and herb types. Artificially contaminated test portions were pre-enriched in each medium and the results were analyzed statistically using Fisher's Exact 2-tailed F test ($p < 0.05$) with pairwise comparisons. There was no difference in recovery of *Salmonella* from curly parsley and basil among the five media ($p > 0.05$). UPB was consistently among the most effective media for recovery of *Salmonella* from the nine produce types; however, *S.* Typhimurium and *S.* Newport were isolated from cabbage more frequently with mBPW than with UPB ($p < 0.05$). Comparisons of the results among the preenrichment media from all experimental trials, with leafy green produce and herbs, demonstrate that *Salmonella* is more effectively detected and isolated using buffered enrichments than with the currently recommended LB ($p < 0.05$). There were no significant differences among the buffered preenrichments for the detection of *Salmonella*-positive test portions of the produce tested (BAX (160 *Salmonella*-positive test portions/480 test portions), UPB (176/480), mBPW (184/480), BPW (169/480), LB (128/480))($p > 0.05$).

1. Introduction

1.1. The need for improved methods for detection of Salmonella contamination in leafy green produce and herbs

Recent estimates on the percentage of foodborne illnesses in the U.S. associated with the consumption of contaminated produce, particularly with leafy greens such as spinach and lettuce, range from approximately 12–14% (Lynch et al., 2009; Jablasone et al., 2012; Boore et al., 2010; Stopforth et al., 2008; Doyle and Erickson, 2008). Compared to estimates prior to 1970s, which were <1%, these figures represent significant increases in the number of produce-related outbreaks during the last several decades (Buck et al., 2003; Lynch et al., 2009). Although these rates may reflect increased consumption of produce, changes in agricultural practices, processing, and packaging of produce may also be contributing factors (Lynch et al., 2009; Sivapalasingam et al., 2004; Olaimat and Holley, 2012; Gil et al., 2015). Contamination of leafy green produce can occur at any phase during the movement of produce within the farm to fork continuum. These include those of production, harvesting, transport, packaging, and preparation for consumption (Olaimat and Holley, 2012). When grown in open fields, leafy greens are exposed to many types of wildlife, including mammalian and avian species, which commonly harbor *Salmonella* in their gastrointestinal tracts and therefore excrete *Salmonella* into the environment (Hanning et al., 2009; Quiroz-Santiago et al., 2009; Liu et al., 2013). Leafy greens can also come into contact with *Salmonella* through contaminated surface water. Heavy rain or flooding may facilitate both the contamination of surface water with pathogenic bacteria and transfer of that contaminated water

* Corresponding author. Microbial Methods Development Branch, Division of Microbiology, Office of Regulatory Sciences, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, 5001 Campus Drive, HFS – 711, College Park, MD 20740, USA.
** Corresponding author.
E-mail addresses: Andrew.Jacobson@fda.hhs.gov (A.P. Jacobson), VikasGill@gmail.com (V.S. Gill), gca6@duke.ed (G. Arce).
[1] Present address: Public Health Program, College of Education and Health Sciences, Touro University California, Vallejo, CA, USA.
[2] Present address: Duke University, Durham, NC, USA.

http://dx.doi.org/10.1016/j.fm.2016.11.006

onto leafy green produce in the field (Liu et al., 2013). Additionally, leafy green produce can become contaminated if, at any point in the farm to fork continuum, it is handled by employees or food preparers who are infected with these pathogens (Gil et al., 2015).

Over the last several decades, produce consumption in the U.S has risen significantly, most likely due to increased consumer awareness and interest in the health benefits associated with raw produce consumption (Sivapalasingam et al., 2004), although ethnic diversification, cultural influences, changing dietary habits, and internationalization of the produce market may also have contributed to these changes (Hoelzer et al., 2012; Sewell and Farber, 2001). Other social changes have increased consumers' reliance on easy-to-prepare meals, salad bars, and ready-to-eat vegetable products, which require less time and effort than traditional home cooking, while still appearing to provide convenient and healthy dietary options (Collins, 1997; Phillips and Harrison, 2005). Increased consumption of produce is possibly associated with the higher frequency of produce-related foodborne infections that have occurred; leafy green produce and herbs, have in particular, been associated with multiple illnesses and outbreaks (Sivapalasingam et al., 2004; Johnston et al., 2005; Liu et al., 2013; Doyle and Erickson, 2008). Multiple causative agents have been responsible for these illnesses, including viruses, protozoa, and bacterial pathogens, although the most frequently identified bacterial pathogens have been *E. coli* O157:H7 and *Salmonella* spp. (Abadias et al., 2008; Quiroz-Santiago et al., 2009; Tauxe et al., 1997).

An important part of reducing the risk of foodborne illnesses associated with leafy greens and herbs is to reliably identify sources of contamination (Buck et al., 2003; Johnston et al., 2005). Once identified, practices associated with or contributory to contamination can be eliminated, reduced, or modified in order to prevent future occurrences (Buck et al., 2003). To reduce the time of preparation for fresh produce, procedures such as cleaning, slicing, and cutting might be performed in fields immediately following harvest rather than transported to an off-field site designed for such purpose (Kozak et al., 2013). This practice may increase the likelihood for internal contamination of produce to occur as natural barriers of produce are disrupted in an environment where the presence of bacterial pathogens is more likely than that of a site that is hygienically maintained (Kozak et al., 2013; Tauxe et al., 1997; Duffy et al., 2005). Similarly, some innovative packaging methods, such as modified atmosphere packaging to extend the shelf life of freshly cut bagged produce, may also increase the risks for outbreaks and infections by creating microenvironments that are favorable to the growth of some bacterial pathogens (Fröder et al., 2007; Sewell and Farber, 2001). Amending such practices can help minimize risks of illnesses related to fresh produce, but surveillance and traceback efforts are built on a foundation of reliable detection/isolation assays.

1.2. The existing culture method for Salmonella on leafy greens

The reference culture method for the detection and isolation of *Salmonella* from foods that is used by the United States Food and Drug Administration (FDA) is described in the *Bacteriological Analytical Manual* (BAM) (FDA, 2015). The BAM *Salmonella* culture method requires preenrichment of food samples in a nonselective medium for 24 ± 2 h. The purpose of preenrichment is to resuscitate low levels of injured *Salmonella* and to allow them to proliferate to detectable levels (Budu-Amoako et al., 1992). By bringing injured *Salmonella* to a healthy physiological state during preenrichment, the *Salmonella* are conditioned for competitive growth during the selective enrichment procedures that follow (Budu-Amoako et al., 1992). Several factors may influence the effectiveness of preenrichment, such as the biological and chemical characteristics of the initial food matrix (e.g., plant associated antimicrobial compounds including polyphenols such as flavonoids and tannins), length of incubation time, preenrichment temperature, composition and level of matrix-associated bacterial competitors, and the formulation of the preenrichment media itself (Coppo and Marchese, 2014; Nam et al., 2004; Worcman-Barninka et al., 2001; D'Aoust et al., 1992). In this study, we have evaluated the relative effectiveness of five preenrichment media for the detection and isolation of *Salmonella* from leafy green produce and herbs.

The leafy green produce types selected for this study were those that had been previously involved with produce-related outbreaks. The leafy green most frequently associated with outbreaks has been Romaine lettuce, although this may be a consequence of the amount of this lettuce produced and the fact that it is consumed at relatively high levels in comparison to other leafy green produce types (Liu et al., 2013; Himathongkham et al., 2007; Delaquis et al., 2007). Spinach was the source of an *E. coli* O157:H7 outbreak in 2006 (Abadias et al., 2008; Neal et al., 2008). Culantro (*Eryngium foetidum*), cilantro (*Coriandrum sativum*), parsley (*Petroselinum crispum*), and basil (*Ocimum basilicum*) are leafy green herbs that have been sources of foodborne bacterial infections (Duffy et al., 2005; Hsu et al., 2006; Golberg et al., 2011). We therefore included these items in our evaluations of the preenrichment media. However, with the exception of basil in pesto, herbs are typically consumed in lower quantities than other leafy green produce types, as their use is primarily to enhance the flavor of main dishes rather than be the principle component (Hsu et al., 2006).

All the preenrichment media selected for this study have been used to isolate *Salmonella* and other common foodborne pathogens from foods. The presence of nutrients and buffering capacity of BAX® System MP Media (DuPont Qualicon, Wilmington, DE) is suited for the growth of *Salmonella* and pathogenic *E. coli* and has been shown to be an effective preenrichment medium for the analysis of environmental samples and some foods (Peng et al., 2011; Wallace et al., 2013). Universal Preenrichment Broth (UPB) was developed for use with foods to isolate both *Salmonella* and *Listeria monocytogenes* and is effective with a variety of food matrices (Hammack et al., 2001; Kanki et al., 2009). Buffered Peptone Water (BPW) and modified BPW (mBPW) have previously been evaluated as preenrichment media with the BAM *Salmonella* culture method, and effective for use with some foods (Wang et al., 2015; Hammack et al., 2006). Lactose broth is the default preenrichment medium recommended by the BAM *Salmonella* culture for the detection of *Salmonella* in foods unless otherwise specified (FDA, 2015a,b). The formulations of the selected preenrichment media are presented in Table 1.

2. Materials and Methods

2.1. Leafy green produce

Fresh leafy green produce (iceberg lettuce, Romaine lettuce, baby spinach, Italian parsley, curly parsley, basil, cabbage, culantro, and cilantro) was purchased from local retail outlets or produce wholesalers. Each produce type (approximately 2500 g) was artificially contaminated with a single *Salmonella* serotype prepared from pure culture. Inoculation and mixing procedures were performed in a biological safety cabinet (Nuaire Inc., Plymouth, MN). The bulk inoculated leafy green produce was stored for approximately 72 h (inoculated on a Friday and evaluated the following Monday) and refrigerated at 2–8° C.

Table 1
Formulation of preenrichment media used in evaluations of the BAM *Salmonella* culture method with leafy green produce and herbs.

Component	Preenrichment media (g/L)				
	BAX[a]	UPB[b]	mBPW[c]	BPW[d]	LB[e]
Pancreatic Digest of Casein	U[f]	5.0	–	–	–
Peptone	U	5.0	–	10.0	5.0
Beef Extract	U	–	5.0	–	3.0
Lactose	U	–	–	–	5.0
Dextrose	U	0.5	–	–	–
Monopotassium phosphate	U	15.0	3.0	1.5	–
Sodium chloride	U	5.0	5.0	5.0	–
Disodium phosphate	U	7.0	7.0	3.5	–
Magnesium sulfate	U	0.25	–	–	–
Ferric ammonium citrate	U	0.1	–	–	–
Pancreatic digest of gelatin	U	–	5.0	–	–
Sodium pyruvate	U	0.2	–	–	–

[a] BAX® System MP Media.
[b] Universal Preenrichment Broth.
[c] Modified Buffered Peptone Water.
[d] Buffered Peptone Water.
[e] Lactose Broth.
[f] Unavailable information due to propriety status.

2.2. Salmonella serotypes

Salmonella enterica serotypes used for this study were obtained from the Division of Microbiology's stock culture collection (Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD) and maintained at room temperature on semi-solid tryptic soy agar (TSA), or cryogenically at −80 °C in trypic soy broth (TSB) and glycerol (20%). Sources of the selected *Salmonella enterica* serotypes were associated with environmental samples and foods (Table 2). Our rationale for using different serotypes in evaluations of the preenrichment media was to evaluate the consistency of *Salmonella* detection in leafy green produce and herbs with a variety of *Salmonella* serotypes. Consistency of media effectiveness among different *Salmonella enterica* serovars and produce types was a primary consideration for the identification of an effective and robust preenrichment medium.

2.3. Preparation of inoculum

Serotypes were subcultured from Brain Heart Infusion agar slants into 100 mL volumes of Brain Heart Infusion Broth and incubated at 35.0 ± 2.0 °C for 20–24 h. Ten-mL of the incubated culture suspension was centrifuged for 10 min at 3000*g* (Jouan Inc., Winchester, VA). The supernatant was pipetted from the cell pellet and this was followed by re-suspension of the cells in an equal volume (10 mL) of Butterfield's Phosphate Buffer (BPB). This wash procedure was repeated two additional times. Following removal of the supernatant from the third wash, the cells were re-suspended in 10 mL BPB. A 10 fold dilution series was prepared from the cell suspensions with 90 mL BPB dilution blanks.

The level of inoculation applied to a bulk quantity of produce or herb was determined by aerobic plate count (APC)(FDA, 2015a,b). A dilution volume of 1.0 mL was used for each pour plate and duplicates were prepared for each dilution level evaluated. The pour plate method was performed within 1 h of produce inoculation. Plate count agar (PCA) was liquefied by heating and tempered to 47.5 °C in a circulating water bath before use. Following preparation of pour plate cultures, the heat tempered agar was allowed to solidify at room temperature. Cultures were then incubated at 35.0 ± 2.0 °C for 18–24 h. Colony counts of cultured dilutions were performed to determine the level of *Salmonella* applied to the produce.

Table 2
Sources of *Salmonella* serotypes selected for use in evaluations of UPB, BAX, mBPW, BPW, and LB preenrichment media with the BAM *Salmonella* culture method to analyze leafy green produce and herbs.

Salmonella serotype	Designation	Source
S. Aba	232,778	culantro
S. Agona	4000H	basil
S. Enteritidis	02–0062	organic spinach
S. Gaminara	24N	orange juice
S. Havana	1254H	bone meal
S. Javiana	2080H	frog legs
S. Michigan	2069H	frog legs
S. Montevideo	1H	whole eggs
S. Muenchen	1501H	feather meal/meat bone meal
S. Negev	26H	thyme
S. Newport	02–0061	cilantro
S. Saintpaul	1090H	cantaloupes
S. Sandiego	408,191	coriander
S. Thompson	2051H	thyme leaves
S. Typhimurium	368,477	tango lettuce

2.4. Inoculation of the produce

The volume of the selected dilution was combined with BPB to produce an inoculum volume of 40 mL. A sterile disposable trigger sprayer (Fisher Scientific, Hampton, NH) was used to deliver the *Salmonella* suspension onto the produce. All inoculation procedures were performed in a biohazard hood to prevent contamination of the laboratory environment during the procedure. Sterile disposable gloves and sleeves were worn during manual mixing of the inoculated produce which was performed aseptically for 15 min to uniformly distribute *Salmonella* throughout the produce.

Levels of contamination of the bulk inoculated leafy green produce and herbs for those trials in which fractional values (25–75% positive) were achieved were typically within the range of 0.1–0.14 cfu/g. Fractionally positive results were necessary to differentiate among the five different media at the limit of detection of one or more of the methods (the use of each medium constitutes an individual method) per internationally recognized microbiological methods validation guidelines (Lombard and Leclereq, 2010; AOAC, 2012; ISO, 2015; FDA, 2015a,b).

2.5. Preparation and storage of the inoculated leafy green produce

Following inoculation and mixing, the contaminated leafy green produce was transferred to a sterile plastic tote (22 by 13 by 8 in. (37,494 cm^3), and covered with sterile aluminum foil taped securely to the edges of the tote to minimize desiccation of the produce during storage at 2–8 °C for approximately 72 h.

2.6. Analysis of leafy green produce and herb test portions

Leafy green produce was removed from refrigeration and transferred from the container to a biochemical hood. The inoculated produce was manually mixed for 15 min using the procedure described above. Preparation of test portions for evaluation of each of the preenrichment media was performed as follows: 20 test portions of 25 ± 0.2 g were randomly acquired from the bulk inoculated leafy green produce or herb by weighing into sterile 500 mL wide mouth Erlenmeyer flasks. The preenrichment media evaluated were BAX (DuPont Qualicon, Wilmington, DE), UPB (Becton, Dickinson and Co., Sparks, MD), BPW (Becton, Dickinson and Co., Sparks, MD), mBPW (3M, St. Paul, MN), and LB (Becton, Dickinson and Co., Sparks, MD). A volume of 225 mL of the preenrichment media was added to each of the 20 test portions

designated for that treatment, and contents were swirled 25 times clockwise and 25 times counterclockwise. Test portions treated with LB were allowed to stand at room temperature for 1 h after which time the pH of the culture medium was measured and adjusted, if necessary, to 6.8 ± 0.2 with 1 N NaOH (FDA, 2015a,b). Buffered enrichments were not pH adjusted. All pre-enrichment cultures were incubated at 35 ± 2 °C for 24 ± 2 h. The BAM *Salmonella* culture method for high microbial load foods was followed thereafter (Andrews et al., 2014). Most Probable Number (MPN) for the level of *Salmonella* in the artificially contaminated leafy greens and herbs was calculated for each experimental trial using the 20 tube single dilution MPN method. MPN analysis was performed for each experimental trial (Table 3)(Blodgett, 2009).

Table 3
Relative effectiveness of selected preenrichment media for the detection of *Salmonella* in leafy green produce and herbs.

Produce type	*Salmonella* positive test-portions/20 test portions examined					
	MPN/g[1]	BAX[2]	UPB[3]	mBPW[4]	LB[5]	BPW[6]
Romaine Lettuce						
S. Newport	33.6	20^{a}	20^{a}	20^{a}	18^{a}	20^{a}
S. Newport	13.7	$15^{a,\ b}$	$15^{a,\ b}$	18^{a}	11^{b}	$15^{a,\ b}$
S. Saintpaul	8.39	14^{a}	11^{a}	12^{a}	9^{a}	11^{a}
		49^{a}	**$46^{a,b}$**	**50^{a}**	**38^{b}**	**$46^{a,b}$**
Italian Parsley						
S. Sandiego	1.18	1^{b}	6^{a}	$3^{a,\ b}$	1^{b}	$3^{a,\ b}$
S. Thompson	1.38	$3^{a,\ b}$	6^{a}	$3^{a,\ b}$	4^{a}	0^{b}
		4^{b}	**12^{a}**	**$6^{a,b}$**	**$5^{a,b}$**	**3^{b}**
Cabbage						
S. Typhimurium	0.70	2^{a}	2^{a}	2^{a}	0^{a}	1^{a}
S. Typhimurium	2.05	$7^{a,b}$	4^{b}	10^{a}	0^{c}	4^{b}
S. Newport	2.56	2^{b}	2^{b}	9^{a}	$7^{a,\ b}$	$6^{a,\ b}$
		$11^{a,b}$	**8^{b}**	**21^{a}**	**7^{b}**	**$11^{a,\ b}$**
Iceberg Lettuce						
S. Muenchen	5.21	7^{a}	8^{a}	7^{a}	8^{a}	11^{a}
S. Montevideo	2.47	10^{a}	7^{a}	5^{a}	0^{b}	7^{a}
		$17^{a,b}$	**$15^{a,b}$**	**$12^{a,b}$**	**8^{b}**	**18^{a}**
Curly parsley						
S. Michigan	5.87	6^{a}	10^{a}	8^{a}	11^{a}	10^{a}
S. Javiana	14.9	14^{a}	16^{a}	13^{a}	17^{a}	17^{a}
		20^{a}	**26^{a}**	**21^{a}**	**28^{a}**	**27^{a}**
Cilantro						
S. Agona	3.7	5^{c}	14^{a}	$12^{a,b}$	$7^{b,c}$	$8^{a,b,c}$
S. Enteritidis	2.6	3^{a}	5^{a}	7^{a}	4^{a}	5^{a}
		8^{b}	**19^{a}**	**19^{a}**	**$11^{a,b}$**	**$13^{a,b}$**
Culantro						
S. Negev	1.0	3^{a}	1^{a}	4^{a}	2^{a}	1^{a}
S. Gaminara	2.11	4^{a}	2^{a}	4^{a}	4^{a}	6^{a}
S. Agona	3.05	7^{a}	9^{a}	11^{a}	0^{b}	8^{a}
		$14^{a,b}$	**$12^{a,b}$**	**19^{a}**	**6^{b}**	**$15^{a,b}$**
Basil						
S. Javiana	2.6	4^{a}	7^{a}	3^{a}	6^{a}	4^{a}
S. Saintpaul	0.67	1^{a}	0^{a}	1^{a}	0^{a}	2^{a}
S. San Diego	0.381	1^{a}	0^{a}	2^{a}	0^{a}	2^{a}
S. Havana	1.1	2^{a}	2^{a}	3^{a}	1^{a}	3^{a}
S. Havana	6.63	10^{a}	8^{a}	12^{a}	7^{a}	12^{a}
		18^{a}	**17^{a}**	**21^{a}**	**14^{a}**	**23^{a}**
Baby Spinach						
S. Aba	6.01	9^{a}	12^{a}	12^{a}	6^{a}	7^{a}
S. Agona	3.7	10^{a}	9^{a}	3^{b}	$5^{a,b}$	$6^{a,b}$
		$19^{a,b}$	**21^{a}**	**$15^{a,b}$**	**11^{b}**	**$13^{a,b}$**
Total[7]		**160^{a}**	**176^{a}**	**184^{a}**	**128^{b}**	**169^{a}**

[1]Most Probable Number estimate ($p < 0.05$).
[2]BAX® System MP Media (DuPont Qualicon, Wilmington, DE).
[3]Universal Preenrichment Broth.
[4]Modified Buffered Peptone Water.
[5]Lactose Broth.
[6]Buffered Peptone Water.
[7]Positive test portions for all experimental trials performed (n/480).
[a,b,c]Values in rows not sharing an alphabetical superscript are significantly different ($p < 0.05$).

2.7. Experimental design and statistical analysis

Results were statistically analyzed using Fisher's Exact 2 tailed F-test for pairwise comparisons of preenrichment media evaluated in experimental trials ($p < 0.05$). Analysis of 100 artificially contaminated test portions were performed in each experimental trial, representing 20 test portions evaluated for each of the preenrichment media (BAX, UPB, mBPW, LB, BPW). At least one experimental trial with acceptable fractional results (25–75% positive) was required for each matrix/serotype combination. Data outside the accepted fractional range were reported and included in the overall statistics for each matrix and the study as a whole.

3. Results

Differences in recovery of *Salmonella* among the media occurred in 9 of the 24 experimental trials (Table 3) ($p < 0.05$). These differences occurred with 7 of the 9 produce types (Romaine lettuce, (1 trial), Italian Parsley (2 trials), cabbage (2 trials), iceberg lettuce (1 trial), cilantro (1 trial), culantro (1 trial), and baby spinach (1 trial)) (Table 3)($p < 0.05$). Differences that occurred in these trials commonly involved those in which LB was less effective than 1 or more of the buffered preenrichment media (Table 3) ($p < 0.05$). We often found BAX, UPB, mBPW, BPW, to be statistically equivalent to one another for the recovery of *Salmonella*, yet more effective than LB ($p < 0.05$) (Table 3).

4. Discussion

Microbial pathogens associated with naturally contaminated produce are stressed due to the presence of competitive microflora, plant-derived antimicrobial compounds, and exposure to the changes in temperature that occur during processing and preparation of produce (Delaquis et al., 2007; Gil et al., 2015; Wiberg and Norberg, 1996). *Salmonella* that occur with produce are often, therefore, physiologically impaired. Injury to *Salmonella* might affect efficiencies observed among the preenrichments due to differences in the resuscitation and growth of *Salmonella* during preenrichment (D'Aoust et al., 1992). While this study is not designed to evaluate the effects of injury on the recovery of *Salmonella*, we aged artificially contaminated produce under refrigeration to mimic conditions that produce might be exposed to during its journey from farm to fork and affect sample preenrichment. This is a common technique for the validation of microbiological methods for the detection of pathogens in foods (AOAC, 2012).

Two or more *Salmonella* serotypes were included for each produce type. The *Salmonella* serotypes used for these evaluations were selected because of their association with foods and agricultural products (Table 3). All *Salmonella enteria* serotypes are potentially pathogenic and all serotypes have the potential to be present in produce. They were randomly assigned to the produce types used for these evaluations.

In trials where differences among the preenrichment media types were evident, UPB was among the most effective media in 7 trials ($p < 0.05$); however, UPB did not appear to be effective for the recovery of *Salmonella* from cabbage (Table 3). In experimental trials with cabbage demonstrating 25–75% sensitivity with 1 or more preenrichments, mBPW was more effective than UPB (Table 3) ($p < 0.05$). The anomalous results with cabbage may be due to the unique chemical composition of that commodity: it contains a variety of sulfur compounds with antimicrobial properties, including methyl methanethiosulfinate and dimethyl trisulfide that may have affected results (Kyung and Fleming, 1997). As shown in Table 3, LB was consistently inconsistent compared to

the other media for the recovery of *Salmonella* across many of the produce types in our study ($p < 0.05$). This might be an effect of the acidic conditions produced during lactose fermentation by competitors: LB does not contain buffers so it does not maintain a stable pH during preenrichment (Table 1). LB has been used as a preenrichment medium for samples thought to be contaminated with *Salmonella* due to *Salmonella's* ability to tolerate slightly higher acidic conditions than many of its competitors (North, 1960). Thus, LB was thought to select for *Salmonella* over its competitors. It was thought that the competitors would ferment lactose, drive the pH down, die off and leave the more acid tolerant *Salmonella* to grow out during selective enrichments and selective/differential plating. Research presented here indicates that this might not be the case.

Unlike LB, UPB, mBPW, and BPW media contain potassium phosphates which are known to provide buffering capacity (Nam et al., 2004; Pikal-Cleland et al., 2002; Antwi et al., 2008). Although the formulation of BAX® System MP Media is proprietary and its precise content is not known, its ability to maintain neutral pH during microbial growth has been recognized (Ganz and Gill, 2013). The relative amounts of monopotassium phosphate in UPB, mBPW, and BPW, suggest that UPB has the highest buffering capacity (Table 1), followed by mBPW, which has twice the amount of phosphate as BPW (Table 1). However these differences in formulation do not seem to have affected our results, since these four buffered enrichments were statistically equivalent for recovery of *Salmonella* from produce (Table 3) ($p > 0.05$).

5. Conclusions

These evaluations demonstrate that buffered preenrichment media is more effective than LB for the isolation and detection of *Salmonella enterica* from artificially contaminated leafy green produce and herbs (Table 3) ($p < 0.05$). BAX, UPB, mBPW, and BPW were equally effective for the analysis of leafy green produce with the BAM *Salmonella* culture method, and all of the buffered enrichments evaluated in this study were significantly more productive than LB for the recovery of *Salmonella* from leafy green produce and herbs (Table 1) ($p < 0.05$).

Acknowledgements

The authors thank Lili Fox Vélez, Ph.D. for scientific writing support.

References

Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. Int. J. Food Microbiol. 123 (1–2), 121–129.

Antwi, M., Theys, T., Bernaerts, K., Van Impe, J., Geeraerd, A., 2008. Validation of a model for growth of *Lactococcus lactis* and *Listeria innocua* in a structured gel system: effect of monopotassium phosphate. Int. J. Food Microbiol. 125 (3), 320–329.

AOAC INTERNATIONAL Official Methods of Analysis 19th Edition Appendix J, 2012. Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces. Retrieved July 1, 2016 from. http://www.eoma.aoac.org/app-j.pdf.

Blodgett, R.J., 2009. Planning a serial dilution test with multiple dilutions. Food Microbiol. 26 (4), 421–424.

Boore, A., Herman, K.M., Perez, A.S., Chen, C.C., Cole, D.J., 2010. Surveillance for foodborne disease outbreaks – United States, 2007. Morb. Mortal. Wkly. Rep. 59 (31), 973–979.

Buck, J.W., Walcott, R.R., Beuchat, L.R., 2003. Recent trends in microbiological safety of fruits and vegetables. Plant Health Prog. http://dx.doi.org/10.1094/PHP-2003-0121-01-RV.

Budu-Amoako, E., Toora, S., Ablett, R.F., Smith, S., 1992. Evaluation of the ability of primary selective enrichment to resuscitate heat-injured and freeze-injured *Listeria monocytogenes* cells. Appl. Environ. Microbiol. 58 (9), 3177–3179.

Collins, J.E., 1997. Impact of changing consumer lifestyles on the emergence/re-emergence of foodborne pathogens. Emerg. Infect. Dis. 4 (3), 471–479.

Coppo, E., Marchese, A., 2014. Antibacterial activity of polyphenols. Curr. Pharm. Biotechnol. 15 (4), 380–390.

Delaquis, P., Bach, S., Dinu, L., 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. J. Food Prot. 70 (8), 1966–1974.

Doyle, M.P., Erickson, M.C., 2008. Summer meeting 2007- the problems with fresh produce: an overview. Soc. Appl. Microbiol. 105 (2), 317–330.

Duffy, E.A., Cisneros-Zevallos, L., Castillo, A., Pillai, S.D., Ricke, S.C., Acuff, G.R., 2005. Survival of *Salmonella* transformed to express green fluorescent protein on Italian parsley as affected by processing and storage. J. Food Prot. 68 (4), 687–695.

D'Aoust, J.-Y., Sewell, A.M., Warburton, D.W., 1992. A comparison of standard cultural methods for the detection of foodborne Salmonella. Int. J. Food Microbiol. 16 (1), 41–50.

FDA, 2015a. *Bacteriological Analytical Manual*, Chapter 3. Retrieved August 26, 2016, from. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm.

FDA, 2015b. *Bacteriological Analytical Manual*, Chapter 5 *Salmonella*. Retrieved July 1, 2016, from. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm.

Food & Drug Administration Office of Foods and Veterinary Medicine, 2015. Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds. Retrieved July 1, 2016, from. http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf.

Fröder, H., Martins, C.G., De Souza, K.L., Landgraf, M., Franco, D., Destro, M.T., 2007. Minimally processed vegetable salads: microbial quality evaluation. J. Food Prot. 70 (5), 1277–1280.

Ganz, K., Gill, A., 2013. Inhibition of polymerase chain reaction for the detection of *Escherichia coli* O157:H7 and *Salmonella enterica* on walnut kernels. Food Microbiol. 35 (1), 15–20.

Gil, M., Selma, M., Suslow, T., Jacxsens, L., Uyttendaele, M., Allende, A., 2015. Pre- and postharvest preventive measures and intervention strategies to control microbial food safety hazards of fresh leafy vegetables. Crit. Rev. Food Sci. Nutr. 55 (4), 453–468.

Golberg, D., Kroupitski, Y., Belausov, E., Pinto, R., Sela, S., 2011. *Salmonella* Typhimurium internalization is variable in leafy vegetables and fresh herbs. Int. J. Food Microbiol. 145 (1), 250–257.

Hammack, T.S., Amaguaña, R.M., Andrews, W.H., 2001. An improved method for the recovery of *Salmonella* serotypes from orange juice using universal preenrichment broth. J. Food Prot. 64 (5), 659–663.

Hammack, T.S., Johnson, M.L., Jacobson, A.P., Andrews, W.H., 2006. Effect of sample preparation and preenrichment media on the recovery of *Salmonella* from cantaloupes, mangoes, and tomatoes. J. AOAC Int. 89 (1), 180–184.

Hanning, I.B., Nutt, J.D., Ricke, S.C., 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne Pathog. Dis. 6 (6), 635–648.

Himathongkham, S., Dodd, M.L., Yee, J.K., Lau, D.K., Bryant, R.G., Badoiu, A.S., Lau, H.K., Guthertz, L.S., Crawford-Miksza, L., Soliman, M.A., 2007. Recirculating immunomagnetic separation and optimal enrichment conditions for enhanced detection and recovery of low levels of *Escherichia coli* O157:H7 from fresh leafy produce and surface water. J. Food Prot. 70 (12), 2717–2724.

Hoelzer, K., Pouillot, R., Egan, K., Dennis, S., 2012. Produce consumption in the United States: an analysis of consumption frequencies, serving sizes, processing forms, and high-consuming population subgroups for microbial risk assessments. J. Food Prot. 75 (2), 328–340.

Hsu, W., Simonne, A., Jitareerat, P., 2006. Fates of seeded *Escherichia coli* O157:H7 and *Salmonella* on selected fresh culinary herbs during refrigerated storage. J. Food Prot. 69 (8), 1997–2001.

ISO (International Organization for Standardization), 2015. EN ISO/FDIS 16140 Microbiology of Food and Animal Feeding Stuffs-Protocol for the Validation of Alternative Methods. Accessible at. http://www.freestd.us/soft/55607.htm.

Jablasone, J., Warriner, K., Griffiths, M., 2012. Interactions of *Escherichia* coli O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. Int. J. Food Microbiol. 99 (1), 7–18.

Johnston, L.M., Jaykus, L., Moll, D., Martinez, M., Anciso, J., Mora, B., Moe, C.L., 2005. A field study of the microbiological quality of fresh produce. J. Food Prot. 68 (9), 1840–1847.

Kanki, M., Seto, K., Jakata, J., Harada, T., Kumeda, Y., 2009. Simultaneous enrichment of shiga toxin-producing *Escherichia coli* O157 and 026 and *Salmonella* in food samples using universal preenrichment broth. J. Food Prot. 72 (10), 2065–2070.

Kozak, G.K., MacDonald, D., Landry, L., Farber, J.M., 2013. Foodborne outbreaks in Canada linked to produce: 2001 through 2009. J. Food Prot. 76 (1), 173–183.

Kyung, K.H., Fleming, H.P., 1997. Antimicrobial activity of sulfur compounds derived from cabbage. J. Food Prot. 60 (1), 67–71.

Liu, C., Hofstra, N., Franz, E., 2013. Impacts of climate change on the microbial safety of pre-harvest leafy green vegetables as indicated by O157 and *Salmonella* spp. Int. J. Food Microbiol. 163 (2–3), 119–128.

Lombard, B., Leclereq, A., 2010. Validation of innovative food microbiological methods according to the EN ISO 16140 standard. Food Anal. Methods. http://dx.doi.org/10.1007/s12161-010-9154-4.

Lynch, M.F., Tauxe, R.V., Hedberg, C.W., 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol. Infect. 137 (3), 307–331.

Nam, H.M., Murinda, S.E., Nguyen, L.T., Oliver, S.P., 2004. Evaluation of universal pre-enrichment broth for isolation of *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* from dairy farm environmental samples. Foodborne

Path. Dis. 1 (1), 37–44.
Neal, J.A., Cabrera-Diaz, E., Marquez-Gonzalez, M., Maxim, J.E., Castillo, A., 2008. Reduction of *Escherichia coli* O157:H7 and *Salmonella* on baby spinach, using electron beam radiation. J. Food Prot. 71 (12), 2415–2420.
North, W.R., 1960. Lactose pre-enrichment method for isolation of *Salmonella* from dried egg albumen. Its use in a survey of commercially produced albumin. Appl. Microbiol. 9, 188–195.
Olaimat, A., Holley, R., 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol. 32 (1), 1–19.
Peng, L.X., Wallace, M., Andaloro, B., Fallon, D., Fleck, L., Delduco, D., Tice, G., 2011. Modification of the BAX system PCR assay for detecting *Salmonella* in beef, produce, and soy protein isolate. J. AOAC Int. 94 (1), 172–178.
Phillips, C.A., Harrison, M.A., 2005. Comparison of the microflora on organically and conventionally grown spring mix from a California processor. J. Food Prot. 68 (6), 1143–1146.
Pikal-Cleland, K., Cleland, J., Anchordoguy, T., Carpenter, J.F., 2002. Effect of glycine on pH changes and protein stability during freeze-thawing in phosphate buffer systems. J. Pharm. Sci. 91 (9), 1969–1979.
Quiroz-Santiago, C., Rodas-Suárez, O.R., Vázquez, Q., Carlos, R., Fernández, F.J., Quiñones-Ramírez, E.I., Vázquez-Salinas, C., 2009. Prevalence of *Salmonella* in vegetables from Mexico. J. Food Prot. 72 (6), 1279–1282.
Sewell, A.M., Farber, J.M., 2001. Foodborne outbreaks in Canada linked to produce. J. Food Prot. 64 (11), 1863–1877.
Sivapalasingam, S., Friedman, C.R., Cohen, L., Tauxe, R., 2004. Fresh Produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. 67 (10), 2342–2353.
Stopforth, J.D., Mai, T., Kottapalli, B., Samadpour, M., 2008. Effect of acidified sodium chlorite, chlorine, and acidic electrolyzed water on *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* inoculated onto leafy greens. J. Food Prot. 71 (3), 625–628.
Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Madden, J., Wachsmuth, K., 1997. Microbial hazards and emerging issues associated with produce: a preliminary report to the national advisory committee on microbiologic criteria for foods. J. Food Prot. 60 (11), 1400–1408.
Wallace, F.M., Andalora, B., Fallon, D., Corrigan, N., Varkey, S., DeMarco, D., Farnum, A., Tadler, M., Hoelzer, S., Weller, J., Davis, E., Rohrbeck, J., Tice, G., 2013. Detection of *Salmonella* species in a variety of foods by the DuPont™ BAX® System real-time PCR assay for Salmonella: first Action 2013.02. J. AOAC Int. 97 (3), 868–875.
Wang, H., Gill, V.S., Ming-Cheng, C., Gonzalez-Escalona, N., Irvin, K.A., Zheng, J., Bell, R.L., Jacobson, A.P., Hammack, T.S., 2015. Evaluation and comparison of rapid methods for the detection of *Salmonella* in naturally contaminated pine nuts using different preenrichment media. Food Microbiol. 46, 58–65.
Wiberg, C., Norberg, P., 1996. Comparison between a cultural procedure using Rappaport-Vassiliadis broth and motility enrichments on modified semisolid Rappaport-Vassiliadis medium for *Salmonella* detection from food and feed. Int. J. Food Microbiol. 29 (2), 353–360.
Worcman-Barninka, D., Destro, M.T., Fernandes, S.A., Landgraf, M., 2001. Evaluation of motility enrichment on modified semi-solid Rappaport-Vassiladis medium (MSRV) for the detection of *Salmonella* in foods. Int. J. Food Microbiol. 64 (3), 387–393.