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Growth of healthy and sanitizer-injured *Salmonella* cells on mung bean sprouts in different commercial enrichment broths

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ABSTRACT

The ability of nine commercial broths to enrich healthy and 90% sanitizer-injured *Salmonella* Typhimurium and *Salmonella* cocktail on mung bean sprouts was evaluated to select an optimum broth for detection. Results showed that *S.* Typhimurium multiplied faster and reached a higher population in buffered peptone water (BPW), *Salmonella* AD media (AD) and ONE broth-*Salmonella* (OB), compared with other broths. Healthy and 90% sanitizer-injured *Salmonella* at low concentrations increased by 4.0 log CFU/ml in these three broths. However, no *Salmonella* growth was observed in lactose broth (LB). Further investigation showed that during incubation, pH of LB dropped from 6.7 to 4.2, due to production of lactic (66 mM) and acetic acids (62 mM) by lactic acid bacteria that were identified as dominant microbiota in bean sprouts. Though no cell membrane damage was detected by propidium monoazide combined with real-time PCR, it was found that LB inhibited *Salmonella* growth, especially from low inoculum levels. This study suggests that in consideration of effectiveness and cost, BPW would be a suitable enrichment broth to use for isolating and detecting *Salmonella* on mung bean sprouts, while using LB might cause false negative results in *Salmonella* detection by either PCR or standard cultural method.

1. Introduction

Mung bean sprouts, which are mainly grown in Asian countries and are used extensively in local cuisine, have been shown to have high nutrient content (Cerna-Cortes et al., 2013; Neo et al., 2013). However, due to the humid and nutrient-rich environment of sprouting, it is now recognized that sprouts can harbor foodborne pathogens, especially *Salmonella* spp. (Harris et al., 2003; Yang et al., 2013). In 2009, 235 persons from 14 states in the United States (US) were infected with *S.* Saintpaul after consuming contaminated alfalfa sprouts (CDC, 2009). In 2011, an international outbreak of 106 clinical cases of *S.* Newport infections due to mung bean sprouts consumption was reported in Germany along with 20 confirmed illnesses in the Netherlands (Bayer et al., 2014). More recently, 111 people from 12 states in US were infected by an outbreak of *S.* Enteritidis linked to mung bean sprouts (FDA, 2014a).

Due to the high risk of sprout borne salmonellosis, the need to improve the efficiency of detecting *Salmonella* presence in sprouts has become a food safety concern of high priority (Yang et al., 2013). Methods to detect *Salmonella* in sprouts as described currently by the U.S. Food and Drug Administration (FDA) include only those developed for use with alfalfa seeds and mung beans (FDA, 2014b). It was also reported later that testing sprouts, as well as spent irrigation water, provide more effective surveillance of sprouts than analyzing sprout seeds (Tortorello and Fu, 2005) since widespread contamination of sprouts might occur during the sprouting phase (FDA, 2004). Usually, *Salmonella* might be present in low concentration or injured conditions in sprouts due to the disinfection treatment on seeds or sprouts after harvesting (Wilderdyke et al., 2004), therefore, enrichment is a critical step in detection of *Salmonella* in sprouts by enabling resuscitation of *Salmonella* cells to detectable levels. Recently, the superiority of a case-specific enrichment media that takes other factors into consideration

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such as food matrix and background microbiota was highlighted (Taskila et al., 2012). This is especially necessary because in Singapore, mung bean sprouts found at retail markets have high levels of background microbiota (8.0 log CFU/g) (Seow et al., 2012), which makes it imperative to identify an effective enrichment broth to recover *Salmonella* among a high level of competitve microorganisms.

To shorten the total detection period, one-step enrichment was usually adopted by rapid detection systems instead of the two-step enrichment that suggested by FDA and ISO (Maks and Fu, 2013; Zheng et al., 2013). There are many commercially available broth media, which have been used for *Salmonella* enrichment. BPW and brain heart infusion (BHI) broth have been evaluated as enrichment media for *Salmonella* in sprout-related studies (Kisluk and Yaron, 2012; Tu et al., 2003). Tetrathionate (TT) broth for selective enrichment and BPW supplemented with novobiocin were also utilized for the detection of *Salmonella* in alfalfa sprouts (Maks and Fu, 2013). Additionally, many media suppliers have developed advanced one-step enrichment broths such as AD from Dupont and OB from Oxoid (Zheng et al., 2013). However, no study has been conducted to compare different commercial enrichment broths in detecting *Salmonella* in mung bean sprouts.

LB is currently used as a pre-enrichment broth for *Salmonella* in analyzing egg-containing products, coconut, meats, leafy green vegetables, alfalfa seeds and mung beans (FDA, 2014b). Because no specific procedures were assigned for mung bean sprouts, most studies have used LB as the enrichment broth for this commodity (Cerna-Cortes et al., 2013; Inami et al., 2001). However, a previous study showed that LB was ineffective in enriching *S.* Typhymurium on raw duck wing samples (Zheng et al., 2013) and the same result with mung bean sprouts was noted in this study. To improve the detection protocol, it is necessary to demonstrate why the growth of *Salmonella* in bean sprouts was not supported by LB. Therefore, the aim of this study was to select a suitable enrichment broth for the detection of *Salmonella* on mung bean sprouts by comparing the growth of healthy and sanitizer-injured *Salmonella* in various broth media using growth modeling. The ineffectiveness of LB in enriching *Salmonella* cells on mung bean sprouts was investigated by comparing pH changes, production of organic acids and cell viability that occurred among the evaluated enrichment broths.

2. Materials and methods

2.1. Bacterial cultures

Prior to experiments, *Salmonella* Typhimurium ATCC 14028 (American Type Culture Collection, Manassas, VA, USA), *S.* Tennessee ATCC 10722, *S.* Agona BAA 707, *S.* Montevideo BAA 710, *S.* Newport ATCC 6962, *S.* Saintpaul ATCC 9712 were cultured twice in 10 ml of tryptone soy broth (TSB; Oxoid, Basingstoke, Hampshire, UK) for 24 h at 37 °C. Each serotype was conditioned to 100 ppm nalidixic acid (NA; Sigma–Aldrich, St. Louis, MO, USA) by successive culturing with increasing concentrations of NA in TSB. Development of resistance to NA enables growth of *Salmonella* from background microbiota in mung bean sprouts.

2.2. Preparation of healthy and sanitizer-injured Salmonella cells

Before inoculation, 1 ml of *S.* Typhimurium (ca. 10^8 CFU/ml) was centrifuged at 3500× g for 10 min at 4 °C, washed twice with 1 ml of 0.1% (w/v) peptone water (PW; Oxoid) and the pellet was resuspended in 1 ml of 0.1% (w/v) PW. Equal aliquot (200 μl) of each *Salmonella* serovar culture, except for *S.* Typhimurium, was aseptically combined, followed by washing with PW as described above, to produce a cocktail of the five serovars with a final concentration of approximately 10^8 CFU/ml.

To prepare sanitizer-injured *Salmonella* cells, sodium hypochlorite solution (household bleach) was selected as the sanitizer (Hygold Chemical Supplies, Singapore). The concentration of free chlorine in sanitizer was determined using RQflex® 10 Reflectoquant® (Merck, Darmsradt, Germany) according to manufacturer's instruction. Different concentrations of active chlorine (200, 100, 50, 25, 2.5 ppm) were prepared by diluting the bleach solution with distilled water (Neo et al., 2013). An aliquot of 100 μl of either *S.* Typhimurium or the *Salmonella* cocktail (ca. 10^8 CFU/ml) was transferred to 10 ml of prepared sanitizer solution to which it was treated for 60, 90, or 180 s at room temperature by gentle mixing for uniform sanitizing. After sanitizer treatment, cells were immediately washed and 10 fold diluted with 0.1% (w/v) PW. The sub-lethal injury of *Salmonella* cells was determined by comparing the counts grown on tryptone soy agar (TSA; Oxoid) as non-selective agar and xylose-lysine-desoxycholate agar (XLD; Oxoid) as selective agar. The percentage of injured cells was calculated by the following formula (Uyttendaele et al., 2008);

$$\text{Sub} - \text{lethal injury}(\%) = \left(1 - \frac{\text{Colonies on XLD}}{\text{Colonies on TSA}}\right) \times 100$$

2.3. Inoculation

Raw mung bean sprouts (*Vigna radiata*) were purchased from a local market in Singapore and were tested for the presence of *Salmonella* using XLD prior to inoculation. Mung bean sprouts were inoculated with *S.* Typhimurium or the *Salmonella* cocktail as follows: 500 g mung bean sprouts was submerged in 2 L of aqueous suspension of either healthy or injured cells for 45 min at room temperature with magnetic stirring to reach a final inoculum levels of 10^2, 10^1 or 10^0 CFU/25g, and were air-dried on sterile plastic trays in a laminar flow biosafety cabinet for 3–4 h (Neo et al., 2013). The inoculated mung bean sprouts were then weighted 25 g into each sterile stomacher bag, and stored at 4 °C overnight which simulates storage condition in a supermarket.

2.4. Growth kinetics of S. Typhimurium in different enrichment broths

Five non-selective enrichment broths, buffered peptone water (BPW; Oxoid), lactose broth (LB; Oxoid), nutrient broth no. 2 (NB; Oxoid), tryptone soy broth (TSB; Oxoid), and universal pre-enrichment broth (UPB; Sigma–Aldrich), and four selective enrichment broths, namely *Salmonella* AD media (AD; DuPont®, Wilmington, Delaware, US), BAX® System MP media (MP; DuPont®), ONE broth-*Salmonella* (OB; Oxoid), and selenite broth (SB; Difco, Detroit, Michigan, US) were evaluated in this study. The broths were prepared according to manufacturers' instructions. Each enrichment broth (225 ml) was poured into a Stomacher® bag containing 25 g of mung bean sprouts inoculated with *S.* Typhimurium at level of 10^2 CFU/ml. Contents were homogenized at 1500 rpm for 1 min using a paddle blender (Silver Masticator, IUL Instruments GmbH, Königswinter, Germany) and incubation at 42 °C for MP, AD and OB, or 37 °C for the remaining broths. Cell growth was monitored by sampling at hourly intervals during a 24 h incubation. The number of viable cells, expressed as log CFU/ml, was plotted against time and growth curves were generated by fitting the data to the equation of Baranyi and Roberts (1994) using DMFit (www.ifr.ac.uk/safety/DMFit). Four growth parameters namely, lag phase duration (LPD), which described the duration that bacteria react to a new environment; maximum growth rate (MGR), which is an intrinsic parameter in a constant environment