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Survival of *Arcobacter butzleri* on vacuum packaged chill stored beef

S. Balamurugan *, Rafath Ahmed, James R. Chambers

Agriculture and Agri-Food Canada, Guelph, Ontario, Canada

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ABSTRACT

The ability of *Arcobacter butzleri* to survive and the effect of initial cell numbers on their survival on vacuum packed chill stored beef were examined. In addition, the effect of natural microflora present on commercial beef on the survival of *A. butzleri* under vacuum packaged chill stored conditions was also examined. The numbers of *A. butzleri* on sterile beef cores stored under vacuum packaged conditions at −1.5 or 4 °C dropped significantly ($P<0.05$) over the time of the study (6 wks). In contrast, survival of *A. butzleri* on commercial vacuum packaged beef (beef with natural microflora) was significantly ($P<0.05$) enhanced, resulting in only 0.3 and 1.3 log cfu cm^{-2} drops in numbers at −1.5 °C and 4 °C, respectively, at the end of 6 wks. Survival of *A. butzleri* on sterile beef cores inoculated with higher initial numbers was significantly ($P<0.05$) higher than on core inoculated with lower initial numbers, while on commercial vacuum packaged beef initial *A. butzleri* numbers had no significant ($P>0.05$) effect on its survival. Cores inoculated with ~10^6 cfu cm^{-2} *A. butzleri*, irrespective of storage temperature or packaging conditions, could be enumerated by direct plating up to day 35. However, cores inoculated with ~10^4 cfu cm^{-2}, irrespective of packaging conditions, *A. butzleri* could be enumerated by direct plating up to 28 and 21 days from cores held at −1.5 and 4 °C, respectively. On commercial vacuum packaged beef, irrespective of initial inoculated *A. butzleri* cell numbers or temperature of storage, *A. butzleri* could be enumerated by direct plating even at 6 wks (end of the study). The presence of inoculated *A. butzleri* on commercial vacuum packaged beef, irrespective of the initial cell numbers had no significant ($P>0.05$) effect on the natural microflora numbers compared to uninoculated controls. These results show that natural microflora on commercial vacuum packaged beef afford enhanced survival of *A. butzleri*, irrespective of their initial cell numbers on the surface of beef stored at industry standard vacuum packaging and chill stored conditions. These findings reinforce the requirement for strict hygienic practices or the implementation of decontamination technologies to ensure safety of beef with respect to this pathogen.

1. Introduction

Arcobacters, formerly known as 'aerotolerant *Campylobacters*', are non-spore forming motile slender-curved, Gram-negative bacteria. They differ from the closely related *Campylobacters* by their ability to grow aerobically and in temperatures below 30 °C (Vandamme et al., 1991). Currently the genus *Arcobacter* has 12 recognized species. However, only 3 species namely *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii* are reported as pathogenic for humans (Mansfield & Forsythe, 2000; Phillips, 2001; Vandenberg et al., 2004). The association of these 3 *Arcobacter* species with human gastroenteritis has been reported in a number of countries (Jiang et al., 2010; Taylor, Kiehlbauch, Tee, Pitarangsi, & Echeverria, 1991; Teague et al., 2010; Vandamme, Pugina, et al., 1992; Wybo, Breynaert, Lauwers, Lindenburg, & Houf, 2004). *A. butzleri* is reported to be the fourth most common *Campylobacter*-like organism isolated from the stool of human patients in Belgium and France (Prouzet-Mauleon, Labadi, Bouges, Menard, & Megraud, 2006; Vandenberg et al., 2004).

In the last decade, *Arcobacter* spp. have been increasingly isolated from healthy and diarrheal feces of food animals and foods of animal origin (De Smet et al., 2011; Lehner, Tasara, & Stephan, 2005; Shah, Saleha, Zunita, & Murugaiyah, 2011; Van Driessche, Houf, Van Hoof, De Zutter, & Vandamme, 2003; Wesley et al., 2000). The prevalence of *Arcobacter* spp. in poultry is the highest and can range between 24 and 96% (de Boer, Tilburg, Woodward, Lior, & Johnson, 1996; Jacob, Lior, & Feuerpfeil, 1993), followed by pork (53%), beef (33 to 40%), water (3 to 50%), and seafoods (40%) (Collado, Guarro, & Figueras, 2009; Ertas, Yusuf, Gonulalan, Guner, & Ulger, 2010; Kabeya et al., 2004; Lehner et al., 2005; Rivas, Fegan, & Vanderlinde, 2004; Van Driessche & Houf, 2007). Most food related cases of *Arcobacter* infections are typically due to *A. butzleri* (Atabay, Corry, & On, 1998; Kiehlbauch et al., 1991; Musmanno, Russi, Lior, & Figura, 1997) although *A. cryaerophilus* and *A. skirrowii* have also been detected (Atabay et al., 1998; Ohlendorf & Murano, 2002).

In the beef industry, vacuum packaging and storage at a strictly controlled temperature of −1.5 °C have extended the shelf life of the product to more than 10 wks and are widely used to store and

* Corresponding author at: Agriculture and Agri-Food Canada, Guelph Food Research Centre, 93 Stone Road West, Guelph, Ontario N1G 5C9, Canada. Tel.: +1 226 217 8091; fax: +1 226 217 8181.
E-mail address: balamurugans@agr.gc.ca (S. Balamurugan).

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export raw beef. These conditions significantly reduce the growth of aerobic spoilage bacteria, but can support the growth of anaerobic and microaerophilic organisms such as psychrotrophic Enterobacteriaceae and psychrotrophic lactic acid bacteria. In 2001, a study investigating the effect of vacuum packing of beef at a strictly controlled temperature of −1.5 °C on the survival of *Campylobacter jejuni* showed no significant changes in the numbers of this pathogen (Dykes & Moorhead, 2001), raising food safety concerns. More recently in 2011, the role of natural meat microflora in enhancing *C. jejuni* survival in vacuum packed beef and pork was reported (Balamurugan, Nattress, Baker, & Dilts, 2011). This study showed that *C. jejuni* survival was significantly enhanced in the presence of natural meat microflora. *A. butzleri*, unlike *C. jejuni*, has the ability to grow under both aerobic and microaerophilic conditions and at temperatures as low as 15 °C (Kjeldgaard, Jorgensen, & Ingmer, 2009) and has higher NaCl (5.0% at 25 °C), pH (5.5 to 8.0) and temperature resistance (D-value = 0.38 to 0.76 min at 55 °C) (D'Sa & Harrison, 2005). This makes *A. butzleri* better suited to survive under vacuum packed conditions. Thus, the objective of the present study was to examine the survival of *A. butzleri* on beef under vacuum packaged conditions at normal meat storage temperatures. In addition, the effect of natural microflora and initial numbers of *A. butzleri* on its survival on commercial vacuum packaged chill stored beef was examined.

2. Materials and methods

2.1. Bacteria and culture media

A five strain *A. butzleri* cocktail containing 1 bovine feces isolate, 1 bovine carcass isolate, 2 retail meat isolates and the ATCC 49616 strain was used in the study. All isolates were confirmed as *A. butzleri* by PCR as described by Houf, Tutenel, De Zutter, Van Hoof, and Vandamme (2000). The bovine and meat isolates used in this study were provided by Dr. Kurt Houf (Ghent University, Faculty of Veterinary Medicine, Department of Veterinary Public Health and Food Safety, Belgium). All isolates were maintained at −80 °C and resuscitated by streaking on tryptic soy agar plates (TSA, Oxoid, Nepean, ON) at 28 °C for 48 h under microaerophilic conditions (5% O_2, 10% CO_2, 10% H_2 and 75% N_2) generated using CampyGen™ (Oxoid) gas generation sachets. An isolated colony was transferred to 75 ml tryptic soy broth (TSB, Oxoid) and incubated under microaerophilic conditions for 48 h at 28 °C. Subsequently, 750 μl of this culture was transferred to 75 ml of TSB and incubated under microaerophilic conditions for 24 h at 28 °C. Cells were harvested by centrifugation and pellets from all 5 isolates were reconstituted (mixed into one suspension) in 0.1% sterile peptone water (same volume as supernatant discarded). This reconstituted culture was used to inoculate meat.

2.2. A. butzleri inoculation and survival on sterile beef

Inoculation and survival of the *A. butzleri* cocktail on sterile beef were performed as described previously (Balamurugan et al., 2011). Briefly, fresh boneless beef loins (*Longissimus dorsi*) of normal muscle quality were obtained from a commercial meat plant. Sterile beef muscle tissue disks (10 cm^2) were aseptically excised from the meat as previously described (Greer & Dilts, 1995). Briefly, the outer surface of each loin was sterilized by immersion in 95% (v/v) ethanol, and then igniting the ethanol to remove excess. That procedure was repeated three times, and then the outer 1 cm of muscle surface was removed to expose sterile, underlying tissue. Disks of tissue 10 cm in diameter, and 5 mm thick, were excised aseptically from the interior portion of the muscle. The pH values of muscle tissue disks were determined using an Accumet AP61 portable pH meter (Fisher Scientific, Ottawa, ON Canada) equipped with a flat surface electrode (Accumet 12-620-289, Fisher Scientific). Each core was placed individually on a styrofoam tray and inoculated with 100 μl volume of the *A. butzleri* cocktail suspension (prepared as described in Section 2.1) to a final level of 10^3–10^4 cfu cm^{-2} (low) or 10^5–10^6 cfu cm^{-2} (high). The bacteria were allowed to adhere for 5 min. The trays with the inoculated or uninoculated control cores were then transferred to 8″ × 6″ commercial barrier bags (oxygen transmission rate of 54 cc $m^{-2} \cdot 24\ h^{-1}$; Winpak Ltd., Winnipeg, MB Canada), vacuum packaged and stored at −1.5 or 4 °C. *A. butzleri* was enumerated over a 6 wk storage time by plating appropriate dilutions on *Arcobacter* selective agar (referred to as Houf agar) (Houf, Devriese, De Zutter, Van Hoof, & Vandamme, 2001) and incubating under microaerophilic conditions at 28 °C for 48 h. Simultaneously, another core was enriched for *Arcobacter* spp. under microaerophilic conditions at 28 °C for 48 h followed by swabbing on *Arcobacter* selective agar to detect the presence or absence of *Arcobacter* spp. as described in Houf et al. (2001). The *Arcobacter* selective agar plates were incubated under microaerophilic conditions for 48 h at 28 °C. Presumptive colonies of *Arcobacter* sp. growing on *Arcobacter* selective agar were confirmed as *A. butzleri* by multiplex-PCR using the primers and conditions described by Houf et al. (2000).

To examine the effect of oxygen permeability through the packaging material on *A. butzleri* survival, the above study was repeated with inoculated and uninoculated cores that were vacuum packaged in oxygen impermeable barrier bags that have very low oxygen transmission rate of 0.4 cc $m^{-2} \cdot 24\ h^{-1}$ (Winpak). These studies were repeated 3 times and final bacterial counts were means of these 3 replications.

2.3. A. butzleri inoculation and survival on commercial vacuum packaged beef

Fresh vacuum packed beef loins were obtained from a commercial beef plant. They were transported to the Guelph Food Research Centre under refrigerated conditions and stored at −1.5 °C overnight. Preparation of beef loin and inoculation, vacuum packaging and enumeration of background bacterial counts on the vacuum packaged pieces of meat were performed as described previously (Balamurugan et al., 2011). Briefly, pieces of meat weighing ~500 g (~750 cm^2) were placed in commercial barrier bags. The pH values of muscle tissue were determined before inoculation. A 7.5 ml volume of *A. butzleri* suspension (prepared as described in Section 2.1 and appropriately diluted using 0.1% sterile peptone water) was inoculated onto each of the pieces leading to a final inoculum of 10^3–10^4 cfu cm^{-2} (low) or 10^5–10^6 (high). The inoculum was massaged onto the pieces before vacuum packing. The vacuum packed pieces of meat were incubated at −1.5 or 4 °C for up to 6 wks. Uninoculated control pieces were similarly processed by adding 7.5 ml of sterile peptone water. Enumeration of *Arcobacter* spp. and background bacterial counts was performed on both the inoculated and uninoculated samples over the 6 wk storage period. *Arcobacter* spp. were enumerated using *Arcobacter* selective agar; Houf agar (Houf et al., 2001), and incubated under microaerophilic conditions at 28 °C for 48 h. Presumptive colonies of *Arcobacter* spp. growing on *Arcobacter* selective agar were confirmed as *A. butzleri* by multiplex-PCR using the primers and conditions described by Houf et al. (2000). Selective media described by Baird, Corry, and Curtis (1987) were used to enumerate *Brochothrix* spp., *Pseudomonas* spp., lactic acid bacteria and Enterobacteriaceae. Total aerobes were enumerated using TSA plates incubated for 48 h at 25 °C. Streptomycin thallous acetate agar (STAA; Oxoid) and cephaloridine fucidin cetrimide agar (CFC; Oxoid) were used to enumerate *Brochothrix* spp. and *Pseudomonas* spp., respectively, by incubating the plates at 25 °C for 48 h. Enumeration of presumptive lactic acid bacteria (LAB) was done on de Man, Rogosa and Sharpe agar (MRSA; Difco) with a 72–96 h anaerobic incubation at 25 °C. Enterobacteriaceae were enumerated by plating on Violet Red Bile Glucose agar (VRBGA; BD-Difco, BD Canada, Mississauga, ON Canada) with 18–24 h anaerobic incubation at 35 °C. Anaerobic conditions were established using a BBL anaerobic gas pack system (BD-BBL, BD Canada). Psychrotrophic bacteria were enumerated by plating on TSA and incubating plates at 4 °C for 10 days.