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BRIEF COMMUNICATION

SURVIVAL CAPACITY OF *Arcobacter butzleri* INOCULATED IN POULTRY MEAT AT TWO DIFFERENT REFRIGERATION TEMPERATURES

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SUMMARY

Arcobacter spp. are emerging enteropathogens and potential zoonotic agents that can be transmitted by food and water, being considered a public health risk. The high isolation rate of these bacteria from poultry products suggests that it may be a major source of human infections. One hallmark for differentiating the genus *Arcobacter* from *Campylobacter* includes their growing capacity at low temperatures (15-30 °C) under aerobic conditions. However, little is known about the population density variation of these bacteria at different refrigeration temperatures. The aim of this study was to determine the survival behavior of two different *Arcobacter butzleri* concentrations (10^4 CFU/mL and 10^7 CFU/mL) inoculated on chicken legs and held at two different refrigeration temperatures (4 and 10 °C) throughout storage time. Results have shown that *A. butzleri* had growing capacity both at 4 and 10 °C. No statistical difference between the survival trends was found for both bacterial concentrations and temperatures tested. This study shows that *A. butzleri* is a robust species with regard to storage temperature, and represents a potential health risk for poultry meat consumers.

KEYWORDS: *Arcobacter*; Survival; Poultry; Refrigeration temperature.

Arcobacter spp. are emerging enteropathogens and potential zoonotic agents that can be transmitted by food and water[1,2]. Since its first isolation in 1977, from aborted bovine and porcine fetuses, it has been implicated in mastitis, infertility, miscarriages and gastrointestinal disorders in animals, and cases of gastroenteritis, bacteremia, endocarditis, and peritonitis in humans[3-5].

Nowadays, this genus consists of 21 species[6] of which *Arcobacter butzleri* is the most prevalent one isolated worldwide from environmental samples, water, different animal species, and retailed meats including beef, pork, lamb, and poultry. Also, it has been found in seafood, unpasteurized milk, and even in cheese samples[7-9]. There have been three reports about this genus in Costa Rica, all in poultry products[10-12].

It is remarkable that in absence of a standard isolation and identification method, the true incidence of this potential pathogen is largely unknown[12]. Yet, the high isolation rate in poultry meat and sub-products suggests that it should be considered a major source of human infections[13]. Moreover, it has been ranked as the fourth most common *Campylobacter* organism isolated from human fecal samples in two independent studies[14]. Nevertheless, its pathogenic properties, virulence factors, and its clinical significance remain to be defined, even though some attempts have been made to know their adhesive properties[14-15] as well as the virulence genes associated[14].

One hallmark for differentiating the genus *Arcobacter* and *Campylobacter* has been its growth capacity at low temperatures (15-30 °C), under aerobic conditions[16] . However, little is known about it in terms of survival, particularly at low temperatures and population density variability. This study aimed to determine the effects on two different refrigeration temperatures over two different *Arcobacter butzleri* concentrations inoculated into poultry meat during its storage time in order to evaluate the potential risk for public health of this product.

On ten different occasions, fifteen raw chicken legs were collected from retail markets in the metropolitan area of *San José*, Costa Rica, from January to June 2013, for a total of 150 samples. Samples were transported at temperatures of 4-6 °C to the Food Microbiology Laboratory, University of Costa Rica, and analyzed within 24 h.

The control strain *A. butzleri* (UACH 001), gently provided by the *Universidad Austral de Chile*, and previously purified, was used for inoculating chicken leg samples. A high (10^7-10^8 CFU/mL) and a low (10^3-10^4 CFU/mL) bacterial concentration inoculum was prepared using sterile peptone water at 0.1%.

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Upon arrival, the chicken legs for inoculation were washed with soap and water, and submerged in a chlorine solution (3 mg/L) for 30 min in order to diminish original bacterial load. Chicken legs used as negative controls were processed immediately without being subjected to any disinfection procedure.

In each occasion, chicken legs were individually packed in sterile plastic bags. Seven chicken legs were inoculated with the high concentration suspension; three of them were incubated at 4 ± 1 °C and the other three at 10 ± 1 °C; the last one was used in order to determine the *Arcobacter* load inoculated. Inoculation procedure was performed by adding 1 mL of the *Arcobacter* suspension prepared to each leg. Extensive massage was done for at least one min. to each leg in order to have a homogenous inoculum. The same process was performed for the other seven legs inoculated with the low concentration suspension. A non-inoculated leg was used as a negative control. Refrigeration temperature was recorded every 10 min with data loggers.

A chicken piece from each incubation group was tested on days 3, 6, and 9 to estimate the number of *A. butzleri* colony forming units (CFU) present.

For determination of the number of colony-forming units present in each sample, decimal dilutions were prepared using sterile peptone water at 0.1% and streaked on blood agar plates, which were incubated aerobically at 35 °C for 48 h. The same procedure was performed for the negative control leg on day 0. From each agar plate, at least five typical *Arcobacter* colonies were counted and confirmed by Gram staining, morphology and oxidase reaction.

Student t test was used in order to compare both groups (high and low bacterial concentration and incubation at 4 or 10 °C). Data loggers' registers were strictly checked for assuring that temperatures during assays were always between 4 ± 1 °C and 10 ± 1 °C.

Data obtained on day 0 was used as the base line to define the strains' growth trends. The media obtained after ten replicates for the low bacterial concentration suspension was 8 x 10^4 CFU/mL, and for the high bacterial concentration suspension 1 x 10^7 CFU/mL. No typical colonies were determined in the chicken legs used as negative controls.

Bacterial counts analyzed in time showed an initial increase in number followed by a decrease (Fig. 1). A reduction of one or two logarithms was noted at both temperatures tested, being more pronounced at 10 ± 1 °C than at 4 ± 1 °C, and independently of the bacterial strain dose used. It is important to highlight that, independently of the refrigeration temperature used, bacterial counts tend to stabilize after day 6 of storage for high bacterial concentration samples , and day 9 for low bacterial concentration ones ; complete reduction has never been achieved during the study period.

No significant differences in the survival trends for both bacterial concentrations and temperatures tested were detected.

Currently, emerging enteropathogens as *Arcobacter* are receiving more attention because of their possible consequences for public health. Water and food products of animal origin have been considered as the main transmission routes of these bacteria[1,17] so that efforts should be made to reduce its presence in the food chain.

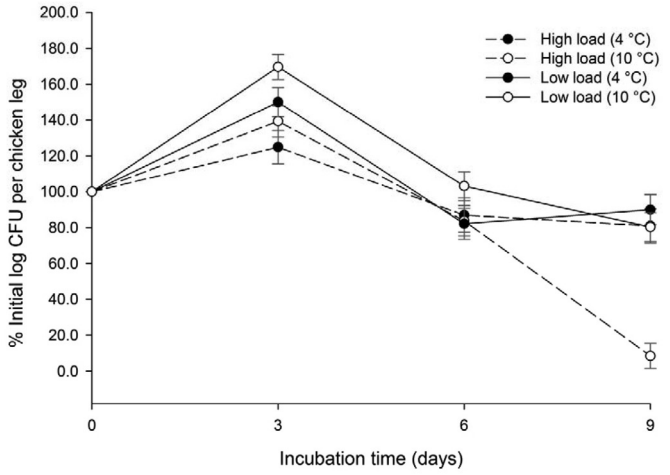

Fig. 1 - Average of 10 different growth counts of *Arcobacter butzleri* (UACH 001) inoculated in chicken legs at different temperatures. Initial bacterial inocula of 1x10^7 CFU/mL were used for high load and of 8x10^4 CFU/mL for low load. No significant difference was observed for both bacterial inocula (critic t value = 3.18 with three degrees of freedom and a two-tail analysis).

The main objective of this work was to determine the behavior of *A. butzleri* at two different refrigeration temperatures frequently used for temporary storage of poultry products.

Results have shown that in all samples evaluated, there was an initial increase in the bacterial count during the first three days of storage, demonstrating *Arcobacter's* growth capacity at refrigeration temperatures. Similar results have been described by different authors, including HILTON *et al.* who described the recovery of these bacteria after 21 days of storage at 4 °C[18]. KJELDGAARD *et al.* have also reported the ability of *A. butzleri* to multiply at 10 °C, and an extended viability, but not growth, at 5 °C in chicken meat juice medium[13]. In contrast, VAN DRIESSCHE & HOUF, in 2008, have reported that some *A. butzleri* strains grew for short periods at 4 and 7 °C in pure drinking water before the bacteria number decreased[10]. Moreover, their results suggest that strain origin does not define its survival capacity, and the presence of organic material influences positively the *Arcobacter* growth at low incubation temperatures (4–20 °C)[17]. The multiplication of *A. butzleri* observed in this work, even at 4 °C on chicken legs, corroborates the observations made by BROWN *et al.*, who have described the contribution of chicken juice to enhance *Campylobacter jejuni* biofilm formation, and as a source of nutrients, a behavior that had not been described for simple media such as *Brucella* broth[19].

A decrease in the number of CFU was observed after day 3 of storage, at both temperatures tested. Aging, nutrients' consumption, and even the accumulation of metabolites can explain this behavior[20].

Nevertheless, bacteria have been detected throughout the period and did not disappear completely. Similar results have been reported by VAN DRIESCHE & HOUF[10] and these authors have reported that *A. butzleri* can be still isolated up to 200 days of incubation in water enriched with organic material at 4 °C and 7 °C.

The survival capacity of *A. butzleri* at low temperatures supports the fact that it can form biofilms. Several researches affirm that biofilms

could be formed at temperatures ranging from 5 to 37 °C, and that these adhesive matrices will protect the bacteria during food processing[13,21].

Taken together, the results of the present study have confirmed that *A. butzleri* is capable of growing at 4 and 10 °C, the storage refrigeration temperatures used for meat products as poultry. Also, that there is no statistically significant difference between the survival trends of the two bacterial inocula tested at both temperatures. Therefore, monitoring of raw meat products for the presence of this emerging pathogen will be of interest to reinforce good agricultural and manufacturing practices through food chain.

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