Antimicrobial effects of *Pseudomonas aeruginosa* on survival of *Campylobacter jejuni* on poultry meat

D.E. CONNER*, M.A. DAVIS and M.L.P. TAM

Department of Poultry Science – Poultry Products Safety and Quality Program, Auburn University, Auburn, AL 36849-5416, USA
***connede@auburn.edu**

Keywords: poultry meat; microbiology; *Campylobacter*; *Pseudomonas*

Summary

In previous research, two isolates of *Pseudomonas aeruginosa* from commercial poultry meat inhibited growth of *Campylobacter jejuni* in laboratory media. Further research was conducted to determine the ability of these *P. aeruginosa* isolates to inhibit the growth of *Campylobacter jejuni* in poultry meat. Three types of poultry meat products (wings, split breast pieces and skinless breast pieces), each representing differences in skin coverage (complete, partial and none, respectively) were used for this research. Equal numbers of the three product types were subjected to one of six bacterial inoculation treatments: uninoculated: *C. jejuni* only, *P. aeruginosa* isolate 1 only, *P. aeruginosa* isolate 2 only, *C. jejuni* + *P. aeruginosa* isolate 1, or *P. aeruginosa* isolate 2. All bacteria were inoculated at 10^4-10^5 cfu/ml. After inoculation, equal numbers of product type were stored for 4 days at 4 vs 10 C in aerobic vs vacuum packaging. After each day of storage, appropriate samples were analyzed to enumerate populations of *C. jejuni* and *P. aeruginosa*. In contrast to results obtained in laboratory medium, the tested isolates of *P. aeruginosa* did not inhibit the growth or survival of *C. jejuni*; although, populations of *C. jejuni* were affected by interactions of product type, packaging type and storage time. The lack of inhibition in poultry meat vs laboratory medium was likely due to high levels on other pseudomonads on the product, which competed with the *P. aeruginosa* inoculum used in this study.

Introduction

In the United States and many other developed countries, the leading pathogen causing acute gastroenteritis is *Campylobacter jejuni* (Butzler and Skirrow, 1979; Blaser *et al.*, 1983; CDC, 2001). Previous studies have shown that this organism can be easily transmitted from the environment to the consumer by poultry products (Hopkins and Scott; 1983, Harris, 1986) and that there is a distinct epidemiological link between the consumption of improperly prepared poultry meat and human illness (Bryan and Doyle, 1995). Even though *Campylobacter* has reservoirs in the environment (Conner *et al.*, 2001), the major reservoir for *Campylobacter* is the intestinal tract of poultry (Oosterom, *et al.*, 1983), especially the ceca and crop (Byrd *et al.*, 1998; Franco and Williams, 2001). Since both of these harbour sites can be ruptured during the initial processing of the chicken carcass, the organism could be transferred to the skin and meat of the carcass. While Davis and Conner (2000) found a relatively low incidence of *Campylobacter* on skinless retail poultry products, they also found that once *Campylobacter* has been introduced onto the skinless product, the bacteria survive very well in the absence of competing microflora (Davis *et al.*, 2002). Since the poultry processing environment is not sterile and there are many other types of bacteria located on poultry skin and meat, Mai (2003) studied the effects of various poultry microbial isolates on the survivability of *C. jejuni*. Results from this study show that many of the psychrotrophic spoilage organisms commonly associated with the poultry carcass would reduce the numbers of *C. jejuni* in both broth and agar cultures by as much as 5.8 $\log_{10}$ cfu/ml (Mai, 2003). The objective of this study was to determine effects of *Pseudomonas* isolates previously determined to inhibit the growth of *C. jejuni* when co-inoculated on various types of poultry products.

Materials and methods

Poultry Products. Portions of broiler meat were used. Wings, skin-on split breast pieces, and skinless, breast pieces were used to provide poultry products representing differing areas of skin

coverage: wings (drumette, flat, tip) - complete skin coverage; skin-on split breast portion - partial and varying skin coverage; and skinless breast meat - zero skin coverage.

Storage Conditions. Two atmospheric conditions, aerobic and vacuum, were used for product storage. Storage temperatures were 4C and 10C for 4 days post-inoculation.

Bacterial Treatments. Sample inoculation consisted of uninoculated product types for control and one of the five following inoculations: *C. jejuni* only, *P. aeruginosa* type 1 only, *P. aeruginosa* type 2 only, *C. jejuni* + *P. aeruginosa* type 1, or *C. jejuni* + *P. aeruginosa* type 2. Three replicates of each product type were inoculated with one of the treatments for day 0 (immediate) testing. Initial populations of *C. jejuni* were $8.5 \times 10^4 - 6.0 \times 10^5$ cfu/ml, and initial populations of *P. aeruginosa* were $1.7 \times 10^4 - 4.8 \times 10^5$ cfu/ml. Inoculum (1 ml) was spread over surface of each piece of poultry product via pipette.

Enumeration and Typing. Populations of *C. jejuni* and *P. aeruginosa* were determined at each day of storage using spiral plate technique with Campy-Cefex and Pseudomonas P agars, respectively. Random colonies from Pseudomonas P plates were then subjected to ribotyping to determine if the *Pseudomonas* colonies were of the same type as the inoculated *Pseudomonas*. Ribotyping was performed using a DuPont Qualicon® Riboprinter using the EcoR1 DNA analysis. Isolate identification was assumed to be correct if the probability was 75% or above.

Data Analysis. Data were arranged in the following manner: total numbers for each product type were 324 (3 samples x 6 inocula x 2 atmospheres x 2 temperatures x 4 sampling days + 36 each for day 0 aerobic testing only). Counts were then converted to $\log_{10}$ values and subjected to Proc GLM and Tukey analyses using the SAS® System. Probability was set at $p \leq 0.05$.

Results and discussion

A summary of the statistical analysis of main effects and interactions of factors on *C. jejuni* populations is given in Table 1. Since there were no *C. jejuni* recovered from treatments not inoculated with *C. jejuni*, statistical analysis was performed only for those treatments where *C. jejuni* was inoculated and, thus, recovered. For surviving *C. jejuni* populations, sample day (day post-inoculation), atmospheric condition, product type and treatment had no significant effect ($p > 0.05$). However, storage temperature did have significant effects ($p \leq 0.001$). *C. jejuni* did not survive as well in the warmer 10C environment as it did in the cooler 4C environment. This suggests that *C. jejuni* may have some adaptive characteristics that allow it to survive at cooler temperatures, and this is also consistent with studies conducted in Norway, in which thermotolerant species of *C. jejuni* survived well at 4C (Franco and Williams, 2001), and it is also known that *Campylobacter* can be cultured from frozen poultry meat (Nachamkin and Blaser, 2000).

Graphical representations of the interactions between main effects on *C. jejuni* populations can be found in Figure 1. For ease of data presentation, the two *P. aeruginosa* treatment values were combined into a single mean for each factor combination, and only data obtained at 4 days of storage are given (Figure 1). While there were significant interactions, these data show there were no specific increasing or decreasing trends within the interactions. Differing trends within each of the interacting variables account for the significant interactions indicated by the statistical analysis. *C. jejuni* survived at a similar rate regardless of *P. aeruginosa* treatment. This is in stark contrast to findings by Mai (2003). Her findings showed that in broth and agar media, the two cultures of *P. aeruginosa* used in this study decreased *C. jejuni* populations by 5.8 logs and 4.6 logs (Mai, 2003). This suggests that although *P. aeruginosa* may affect the survival of *C. jejuni* in isolated populations, the microbial dynamic of poultry meat may not lend itself to this phenomenon.

In this experiment, populations of *Pseudomonas* exceeded 10^7 cfu/ml on all products after one day of storage (data not shown), and there were no differences in populations on product inoculated with Type 1 or Type 2 *P. aeruginosa* vs populations of pseudomonads on uninoculated (control) products. The observation of no difference in treatments that were not inoculated with *P. aeruginosa* and those that were suggests that there were substantial populations of other pseudomonads and similar spoilage organisms already present on the products used. Ribotyping of the selected isolates from the Pseudomonas P agar confirms the presence of a variety of bacteria with growth characteristics similar to *P. aeruginosa*. Isolates of *Serratia, Acinetobacter, Stenotrophomonas* and *Hafnia* were found. Three types of *P. aeruginosa* (including the two inocula) and 2 types of *P. fluorescens* were also isolated. These findings suggests that there is a very complex microbial ecology existed on the tested

poultry skin and meat, and that the two isolates of *P. aeruginosa* used for this study may not have competed well in this environment.

In conclusion, *C. jejuni* survived well in presence of high levels of *P. aeruginosa* and natural spoilage bacteria in the range of products tested here. High levels of these naturally occurring bacteria likely out competed the added *P. aeruginosa*, which either prevented or masked the inhibitory effect seen earlier in laboratory media.

References

Blaser, M. J. Taylor, D. N. and Feldman, R.A. (1983) Epidemiology of *Campylobacter jejuni* infections. *Epidemiol. Rev.* **5**:157-176.

Bryan, F.L. and Doyle, M. P. (1995) Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry, *J.Food Prot.* **58**: 326

Butzler, J. P. and Skirrow, M. B. (1979) *Campylobacter enteritis. Clin. Gastroenterol.* **8**:737-765

Byrd, J. A., Corrier, D. E., Hume, M. E., Bailey, R. H., Stanker, L. H. and Hargis, B. M. (1998) Incidence of *Campylobacter* in crops of preharvest market-age broiler chickens. *Poult. Sci.* **77:** 1303 – 1305

Centers for Disease Control (CDC) and Prevention (2001) Preliminary FoodNet Data on the Incidence of Foodborne Illness --- Selected Sites, United States, 2000. *Morbidity and Mortality Weekly Report (MMWR)*. **50(13**): 241 – 246

Conner, D. E., Davis, M. A. and Zhang, L. (2001) Poultry-borne pathogens: plant considerations, pp. 137-158. *IN* Sams, A. R., (ed.). *Poultry Meat Processing*. CRC Press, Boca Raton, Florida.

Davis, M. A. and Conner, D. E. (2000) Incidence of *Campylobacter* from raw, retail poultry products. *Poult. Sci*. **79 (Suppl. 1**): 54

Davis, M. A. and Conner, D. E. (2002) Factors Affecting Campylobacter Incidence on Various Poultry Meat Products. pp. 446-449, *IN* Conference Proceedings – 7th WPSA Asian Pacific Federation Conference/12th Australian Poultry and Feed Convention.

Franco, D. A. and Williams, C. E. (2001) Campylobacter jejuni, pp. 83-106. *IN* Y. H. Hui, M. D. Pierson, and J. R. Gorham, (eds.). *Foodborne Disease Handbook*, Second Edition, Revised and Expanded. Marcel Dekker, Inc. New York, New York

Harris, N. V., Weiss, N. S., and C. M. Nolan, C. M. (1986) The role of poultry and meats in the etiology of *Campylobacter jejuni/coli* enteritis. *Am. J. Publ. Health* **76**:407-411

Hopkins, R. S. and Scott, A. S. (1983) Handling raw chickens as a source for sporadic *Campylobacter jejuni* infections. *J. Infect. Dis.* **148**: 770 – 772

Mai, T. L. (2003) *Inhibition of Campylobacter jejuni by Bacteria Isolated from Broiler Deboning Operation*. M. S. Thesis, Auburn University. 83 pp.

Nachamkin, I. and Blaser, M. J. (2000) *Campylobacter* 2nd edition. ASM Press, Washington, D. C.

Oosterom, J., Notermans, S., Karman, H. and Engels, G. B. (1983) Origin and prevalence of *Campylobacter jejuni* in poultry processing. *J. Food Prot.* **46**:339-344

Table 1 Effect of Experimental Variables (Factors) on Populations of *C. jejuni*.

	Mean* C. jejuni *Population* (log_{10} *cfu/ml)	***Minimum Significant Difference* (α *= 0.05)***
Sample Day (D)	*NS*[1]	*0.1544*
0	*3.13*	
1	*3.18*	
2	*3.10*	
3	*3.15*	
4	*3.23*	
Storage Temperature (S)	***	*0.064*
4C	*3.37*[a]	
10C	*2.94*[b]	
Treatment (T)	*NS*	*0.1091*
C. jejuni *only*	*3.14*	
C. jejuni + P. aeruginosa *type 1*	*3.19*	
C. jejuni + P. aeruginosa *type 2*	*3.15*	
Atmospheric Condition (A)	*NS*	*0.0748*
Aerobic	*3.18*	
Vacuum Packaged	*3.14*	
Product Type (P)	*NS*	*0.109*
Wing	*3.19*	
Skin-on Split Breast	*3.17*	
Skinless Breast	*3.15*	
Interactions		***Pooled SEM***
S x T	*	*0.0330*
T x A	*	*0.0330*
D x S x T	***	*0.0738*
D x S x A	*	*0.0603*
D x T x P	***	*0.0904*
S x T x P	***	*0.1278*
T x A x P	*	*0.0572*
D x S x T x A	**	*0.1044*

[1]NS = Not Significant ($p > 0.05$), * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Differing superscripts indicate significant differences. Significant differences by column and main effect only.

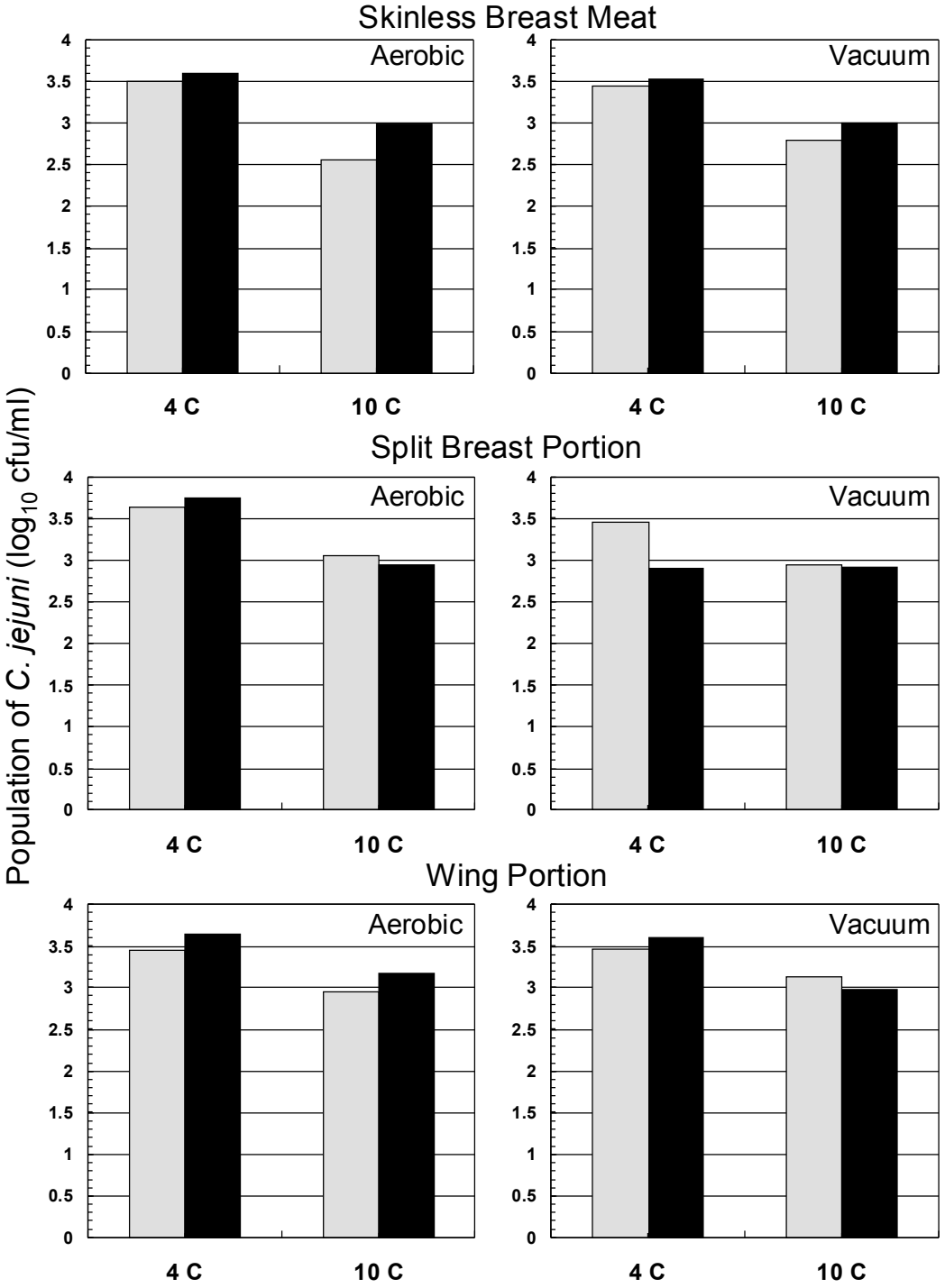

Figure 1. Population of *C. jejuni* on various poultry meat products inoculated with *C. jejuni* only (light bar) or with *C. jejuni* + *P. aeruginosa* (dark bar), then held for 4 days at 4 or 10C in aerobic or vacuum packaging.