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Inhibition of *Campylobacter jejuni* in Chicken by Ethanol, Hydrogen Peroxide, and Organic Acids

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Abstract Growth inhibition of *Campylobacter jejuni* ATCC 33291 was observed in the presence of various preservatives at various temperatures. The addition of ethanol (0.5% to 5%), hydrogen peroxide (0.05%), acetic acid (1%), propionic acid, benzoic acid, and sorbic acid showed strong antibacterial activities against *C. jejuni* at pH 5.5 or 6.5. The addition of 1% acetic acid and lactic acid were most effective at 42 °C, followed by 25°C and 4°C. This indicated that the inhibitory effect was temperature dependent. In the chicken model system, the practical death rate of *C. jejuni* in the FBP-media with 1% acetic acid was much lower than the theoretical decimal rate at all temperatures (4°C, 25°C, and 42°C). Therefore, precaution has to be taken in the use of organic acids as a disinfectant in the chicken slaughterhouse.

Key words: *Campylobacter jejuni*, inhibition, organic acids, chicken

Campylobacter jejuni is an important foodborne pathogen which cause acute bacterial gastroenteritis in humans [2, 5, 10, 19, 20, 21]. Currently, the outbreak of campylobacteriosis has dramatically risen to the point that it exceeds the outbreak of salmonellosis [8]. Vector foods associated with such outbreaks include chicken, milk, and under-cooked poultry. *C. jejuni* is particularly associated with raw chicken [5, 20]. Therefore, the proper use of a safe antimicrobial agent in chicken processing plant for the establishment of Hazard Analysis Critical Control Points (HACCP) is essential.

It is well known that organic acids have a strong antimicrobial effect in reducing the number of pathogens in freshly slaughtered meat carcasses. Lactic acid and acetic acid are suitable candidates since they are known as Generally Recognized as Safe (GRAS). Netten *et al.* [13] stated that the addition of lactic acid caused an 'immediate death' and that the antimicrobial activity should be described as a 'disinfection' rather than 'preservation.' Many studies [1, 12-16] have been conducted on the antimicrobial activity of organic acids on pathogens including *C. jejuni*. However, most of the studies have been carried out at relatively high temperatures, i.e., 20–52°C, rather than at refrigeration conditions [3, 9, 11].

In order to select a suitable disinfectant against *C. jejuni*, we examined the antimicrobial activities of various substances, including ethanol, hydrogen peroxide, and organic acids. Among them, acetic acid was chosen due to its stable antibacterial activity at various temperature, in contrast to lactic acid. Furthermore, acetic acid is safely used as a preservative in food processing. The data obtained here will provide crucial information for the control of *C. jejuni* in poultry processing.

Materials and Methods

Strains and Culture Conditions

Campylobacter jejuni ATCC 33291 was maintained in freeze-dried ampoule and kept at −70°C. The strain was microaerobically cultured in FBP Supplemented Brucella Broth (FBP-SBB) [17, 18] at 42°C for 48 h in an anaerobic jar with a campylobacter microaerophilic system (Difco). The FBP-SBB consisted of 0.9 mM ferrous sulfate, 1.3 mM sodium meta bisulfite, and 2.3 mM sodium pyruvate in Brucella Broth (Difco). Filtered antibiotics (15 mg of vancomycin, 5 mg of trimethoprim lactate, 20,000 IU of polymyxin B, 50 mg/l of cycloheximide) and 3% bovine calf serum (Hyclone, Logan, Utah, U.S.A.) were separately added into FBP-SBB after autoclaving. Either FBP-SBM [FBP-SBB with 2% agar plus 5% defibrinated sheep blood (Komed Co.,

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Bundang, Korea)] or campylobacter selective agar (Lab. M, Co.) were used for the solid culture.

Studies in Media Broth

FBP-SBB was used for all experiments in broth. Predetermined concentrations of ethanol and hydrogen peroxide, 0.05%–5% and 0.001%–0.5%, respectively, were added to the sterile media broth. One % and 2% of various organic acids (acetic acid, propionic acid, lactic acid, sodium benzoate, citric acid, potassium sorbate) were added to FBP-SBB, and the pH of the broth was adjusted to 5.5 or 6.5 by 1 N NaOH before autoclaving. One and a half ml of 10^8 CFU/ml of *C. jejuni* suspension was inoculated to the broth at each condition. The inoculated broth was incubated at 4°C, 25°C, and 42°C. At time intervals, a portion of broth was taken and plated on the campylobacter selective agar and microaerobically incubated for 48 h at 42°C. The acids used were purchased from Sigma. Other products used in this experiment were of reagent grade.

Studies with Chicken Model System

Decimal death rate (D value) with 1% (v/v) acetic acid was estimated at 4°C, 25°C, and 42°C. FBP-SBB with 1% acetic acid was inoculated with a final concentration of approximately 10^7 CFU/ml of *C. jejuni* before the suspension was incubated at 4°C, 25°C, and 42°C. At 30 sec, 5 min, 30 min, and 60 min, the number of reduced cells was checked by the plate count method. Each D value was obtained as D_4=1,320 sec, D_{25}=510 sec, D_{42}=84 sec from initial CFU/ml, [No] to 0.1 No in the linear part of the death curves.

For the model system, chicken purchased from a local supermarket was minced, and 10 g of the sample containing skin and other meat parts was inoculated with 10^8 CFU/g of *C. jejuni*. The inoculated chicken was soaked in 90 ml of 1% acetic acid solution (89 ml of FBP-SBB and 1 ml of acetic acid) in a 250-ml Erlenmeyer flask. The chicken in 1% acetic acid solution was incubated at 4°C, 25°C, and 42°C in waterbaths and the reduction of cell number was checked from 1 D to 15 D values by the plate count method.

Results and Discussion

Inhibition of *C. jejuni* by Various Antimicrobial Agents

In order to observe the response of *C. jejuni* to antimicrobial agents in general, inhibition profiles of *C. jejuni* by ethanol and hydrogen peroxide were checked in the media broth inoculated with 10^4 CFU/ml (low concentration) and 10^7 CFU/ml (high concentration) of the fully grown *C. jejuni* cells (Table 1). The concentrations of ethanol and H_2O_2 were 0.05% to 5% and 0.001% to 0.5%, respectively. Addition of ethanol dramatically inhibited the growth of *C. jejuni* in the low numbered sample at the concentration over 0.5%. After 5 days of incubation, no colonies were detected in the broth of 5% ethanol added. The effect of H_2O_2 was prominent at the concentration of 0.05% to 0.5%, however, it was not effective at low concentration, i.e., 0.05% and 0.01%.

Table 1. Inhibition of the growth of *C. jejuni* ATCC 33291 by the addition of ethanol and hydrogen peroxide at pH 6.5.

CFU/ml

Incubation time		1 day		5 days	
Control		4.8×10^7	2.1×10^4	2.0×10^7	1.4×10^8
EtOH	0.05%	7.8×10^7	3.0×10^4	2.4×10^7	1.8×10^8
	0.1%	5.0×10^7	7.0×10^4	4.4×10^7	4.1×10^7
	0.5%	8.8×10^7	5.0×10^1	1.5×10^7	2.0×10^7
	1%	4.3×10^7	*<10	2.1×10^6	3.4×10^7
	5%	2.4×10^5	<10	<10	<10
H_2O_2	0.001%	8.8×10^7	3.7×10^4	3.8×10^7	3.2×10^7
	0.01%	1.0×10^8	2.0×10^4	3.6×10^7	2.5×10^7
	0.05%	<10	<10	NT**	NT
	0.1%	<10	<10	NT	NT
	0.5%	<10	<10	NT	NT

Initial number of cells: 3.8×10^7 CFU/ml and 2.8×10^2 CFU/ml. *<10: No colonies on the plate inoculated with 0.1 ml of undiluted sample. ** NT : Not tested.

Table 2. Inhibition of the growth of *C. jejuni* ATCC 33291 by the addition of several organic acids.

Acids			Incubation time		
			1 day	2 days	5 days
Control	0	pH 5.5	7.8×10^7	1.2×10^8	5.2×10^8
		pH 6.5	1.1×10^8	8.0×10^8	8.0×10^7
Acetic	1%	pH 5.5	4.2×10^3	*<10	NT**
		pH 6.5	1.1×10^6	<10	NT
	2%	pH 5.5	<10	<10	NT
		pH 6.5	1.6×10^5	<10	NT
Propionic	1%	pH 5.5	<10	<10	NT
		pH 6.5	1.8×10^6	9.0×10^2	<10
	2%	pH 5.5	<10	<10	NT
		pH 6.5	1.3×10^6	<10	NT
Benzoic	1%	pH 5.5	<10	NT	NT
		pH 6.5	<10	NT	NT
	2%	pH 5.5	<10	NT	NT
		pH 6.5	<10	NT	NT
Citric	1%	pH 5.5	1.0×10^6	<10	NT
		pH 6.5	1.4×10^6	2.5×10^7	NT
	2%	pH 5.5	<10	NT	NT
		pH 6.5	3.4×10^5	<10	NT
Sorbic	1%	pH 5.5	<10	NT	NT
		pH 6.5	<10	NT	NT
	2%	pH 5.5	<10	NT	NT
		pH 6.5	<10	NT	NT

CFU indicate colony forming unit. Initial number of cells: 1.4×10^7 CFU/ml. *<10: No colonies on the plate inoculated with 0.1 ml of undiluted sample. **NT: Not tested.

Table 2 shows the antimicrobial effect of acetic acid, propionic acid, benzoic acid, citric acid, and sorbic acid at various conditions. The growth of *C. jejuni* were all suppressed at a certain level. Benzoic acid, sorbic acid, and acetic acid were the most effective, whereas propionic acid and citric acid were the least effective. The acetic acid was finally chosen for further studies, because it has several advantages over ethanol and hydrogen peroxide; 1) it is GRAS, 2) the concentration of ethanol is too high (5%) for practical application, and 3) hydrogen peroxide has to be removed from the final product in food processing.

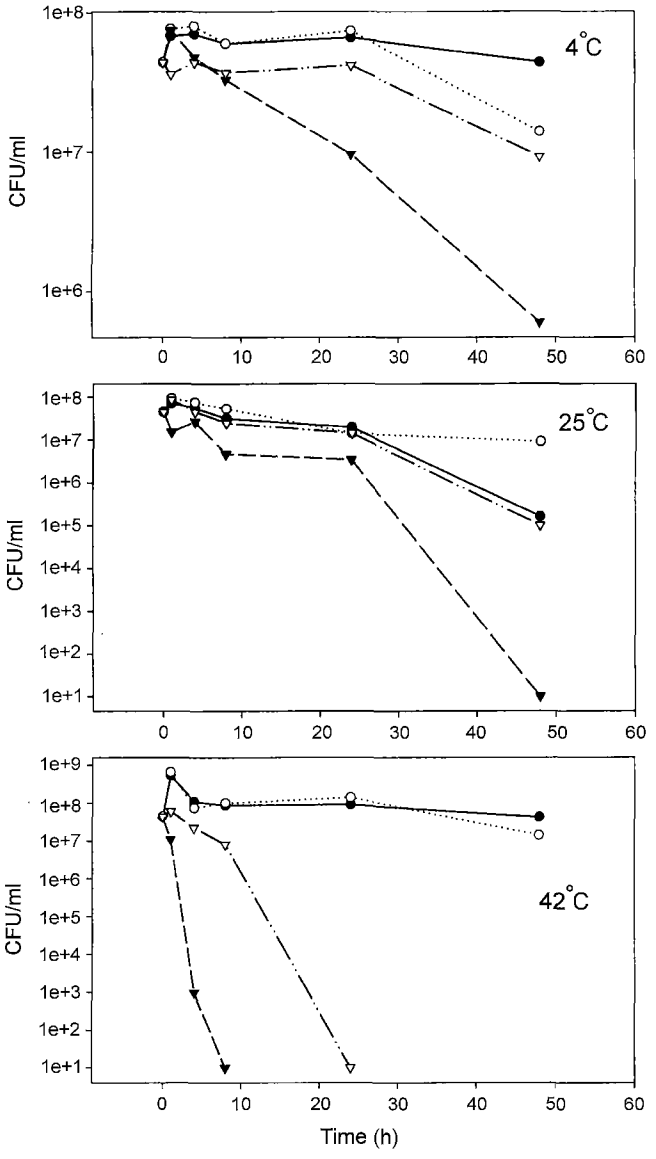

Fig. 1. Inhibiton of *C. jejuni* ATCC 33291 in FBP-supplemented Brucella broth in the presence of 1% acetic acid at three different temperatures.
-●- Control pH 5.5; -○- Control pH 6.5; -▼- Acetic acid 1%, pH 5.5; -▽- Acetic acid 1%, pH 6.5.

The effect of 1% acetic acid at pH 5.5 was stronger than that of 2% at pH 6.5. It is well known that the concentration of undissociated acid plays a critical role in antimicrobial activity [16]. The concentration of undissociated form in 1% acetic acid (167 mM) at pH 5.5 was calculated as 25.7 mM, whereas 2% of acetic acid at pH 6.5 (334 mM) had only 4.8 mM of acetic acid undissociated.

Inhibition of *C. jejuni* by 1% Acetic Acid and Lactic Acid

As shown in Fig. 1, the addition of 1% acetic acid and incubation at 42°C decreased the number of *C. jejuni* from 10^7 CFU/ml to below 10^1 CFU/ml within 24 h at pHs 5.5 and 6.5, while it remained considerable at 4 and 25°C (10^7

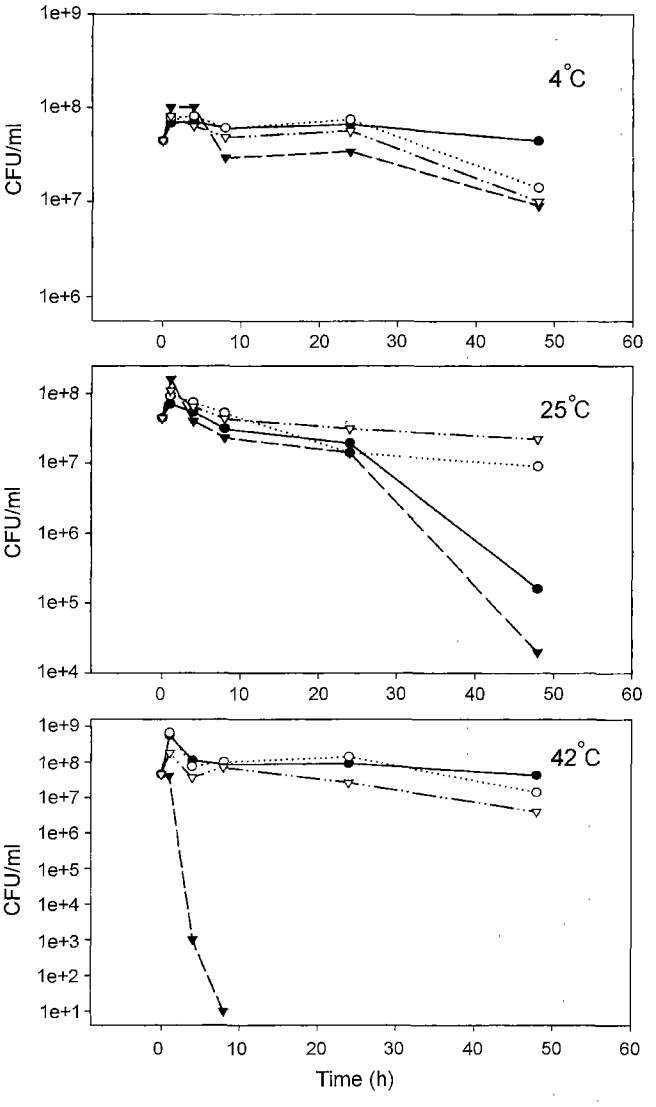

Fig. 2. Inhibiton of *C. jejuni* ATCC 33291 in FBP-supplemented Brucella broth in the presence of 1% lactic acid at three different temperatures.
-●- Control pH 5.5; -○- Control pH 6.5; -▼- Lactic acid 1%, pH 5.5; -▽- Lactic acid 1%, pH 6.5.

and 10^6 CFU/ml remaining at pH 6.5 and pH 5.5 for 24 h, respectively). Lactic acid, however, showed a different pattern (Fig. 2): The degree of inhibition by 1% lactic acid was lower than the case of 1% acetic acid at all temperatures tested. In particular, the inhibition of *C. jejuni* at 4°C by 1% lactic acid was almost unnoticeable, whereas the number of *C. jejuni* was remarkably reduced by the addition of 1% acetic acid under the same condition (Figs. 1 and 2). Considerable resistance of *C. jejuni* was observed by adding of 1% lactic acid at pH 6.5 at 25°C and 42°C. This result did not agree with other reports [12, 13]. Therefore, it was necessary to study further the survival effect of *C. jejuni* in the presence of organic acids under various conditions. According to these results, it was thought that acetic acid was the most reasonable preservative in terms of its effectiveness and safety, and it was applied in the next experimental model system with chicken.

Survival of *C. jejuni* in Model System with Chicken

Figure 3 shows the comparison of the death rate of *C. jejuni* in the chicken model system and the one which was predicted from the media broth without chicken. The death rate of the model system with chicken was lower than the theoretical system. However, the pH for both systems was around 3.9. Therefore, the protection or matrix effect of chicken debris could reduce the effectiveness of a low pH. In other words, chicken debris may facilitate in the repair of the sublethally injured *C. jejuni* cells in the system with chicken [16].

This result showed that the decimal death rate of *C. jejuni* which is related to acetic acid was not in agreement with the theoretical prediction by the condition without chicken. An extended exposure time or increased concentration of acetic acids would assist the proper application to kill *C. jejuni*. However, a high concentration of acetic acid would cause chicken to be leathery or discolored. Even with such demerit, the information of inconsistency of the death rate of *C. jejuni* with acetic acid with or without chicken in the media provides additional knowledge needed to establish HACCP in chicken slaughterhouses.

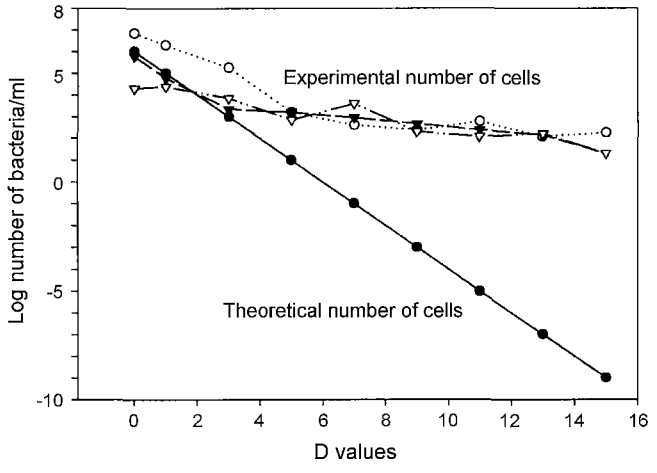

Fig. 3. Comparison of theoretical (-●-) and experimental (-▼-: 4°C; -▽-: 25°C; -○-: 42°C) numbers of *C. jejuni* ATCC 33291 in the model system with 10% chicken and 1% acetic acid.
Cells were exposed under D_4=1,320 sec, D_{25}=510 sec, and D_{42}=84 sec for each 15 decimal rate.

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References

1. Anderson, M. E. 1990. Reducing microbial populations on beef tissues: Concentration and temperature of lactic acid. *J. Food Safety* **12:** 181–190.
2. Barrell, R. A. E. 1981. The survival of *Campylobacter coli/jejuni* in unpasteurised milk. *J. Infect.* **3:** 348–352.
3. Bae, E. A., D. H. Kim, and M. J. Han. 2000. Anti-*Helicobacter pylori* activity of *Bifidobacterium* spp. *J. Microbiol. Biotechnol.* **10:** 532–534.
4. Black, R. E., M. M. Lavine, M. L. Clements, T. P. Hughes, and M. J. Blaser. 1988. Experimental *Campylobacter jejuni* infection in humans. *J. Infect. Disease* **157:** 472–479.
5. Bryan, F. L. and M. P. Doyle. 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *J. Food Prot.* **58:** 326–344.
6. Doyle, M. P. and D. J. Roman. 1982. Response of *Campylobacter jejuni* to sodium chloride. *Appl. Environ. Microbiol.* **43:** 561–565.
7. Doyle, M. P. and D. J. Roman. 1982. Sensitivity of *Campylobacter jejuni* to drying. *J. Food Prot.* **45:** 507–510.
8. Griffiths, P. L. and R. W. A. Park. 1990. Campylobacters associated with human diarrhoeal disease. A review. *J. Appl. Bacteriol.* **69:** 281–301.
9. Hwang, C. A. and L. R. Beuchat. 1995. Efficacy of a lactic acid/sodium benzoate wash solution in reducing bacterial contamination of raw chicken. *Int. J. Food Microbiol.* **27:** 91–98.
10. Lee, Y. H., J. H. Lee, H. J. Cho, E. J. Shin, J. W. Park, and J. H. Park. 1999. Characterization of campylobacters newly isolated from swine gastric mucosa. *J. Microbiol. Biotechnol.* **9:** 778–783.
11. Lee, Y. H., E. J. Shin, J. H. Lee, and J. H. Park. 1999. *Lactobacillus acidophilus* inhibits the *Helicobacter pylori* adherence. *J. Microbiol. Biotechnol.* **9:** 794–797.
12. Leora, A. S. and Y. Qian. 1991. Growth suppression of *Listeria monocytogenes* by lactates in broth, chicken, and beef. *J. Food Prot.* **54:** 283–287.
13. Netten, P. V., J. H. J. Huis in 't Veld, and D. A. A. Mossel. 1994. The immediate bactericidal effect of lactic acid on meat-borne pathogen. *J. Food Prot.* **77:** 490–496.

14. Okrend, A. J., R. W. Johnston, and A. B. Moran. 1986. Effect of acetic acid on the death rates at 52°C of *Salmonella newport*, *Salmonella typhimurium* and *Campylobacter jejuni* in poultry scald water. *J. Food Prot.* **49:** 500–503.
15. Perales, I. and M. I. Garcia. 1990. The influence of pH and temperature on the behaviour of *Salmonella enteritidis* phage type 4 in home-made mayonnaise. *Lett. Appl. Microbiol.* **10:** 19–22.
16. Ray, B. 1996. Control by low pH and organic acids. pp. 409–416. *In: Fundamental Food Microbiology.* CRC Press. Boca Raton, U.S.A.
17. Shin, S. Y., J. H. Park, and W. J. Kim. 1999. Specific detection of enteropathogen *Campylobacter jejuni* in food using a polymerase chain reaction. *J. Microbiol. Biotechnol.* **9:** 184–190.
18. Shin, S. Y., K. Y. Kim, and J. H. Park. 1998. Survival of *Campylobacter jejuni* under aerobic condition. *Kor. J. Food Sci. Technol.* **30:** 916–923.
19. Smibert, R. M. 1984. Genus *Campylobacter.* Sebald and Veron 1963, 907. pp. 111–118. *In* Krieg N. R. and J. G. Holt (eds.), *Bergey's Manual of Systematic Bacteriology*, vol. **1**. Williams and Wilkins Co., Baltimore, U.S.A.
20. Smith, J. L. 1995. Arthritis, Guillani-Barre syndrome, and other sequelae of *Campylobacter jejuni* enteritis. *J. Food Prot.* **58:** 1153–1170.
21. Tenover, F. C. and C. L. Fennell. 1991. The genera *Campylobacter* and *Helicobacter*, pp. 3488–3511. *In* Balows, A., H. G. Truper, W. Harder, and K. H. Schleifer (eds.), *The Procaryotes*, vol. **IV.** 2nd ed. Springer-Verlag, New York, U.S.A.