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Antibacterial Effect of the Lactoperoxidase/Thiocyanate/Hydrogen Peroxide System Against Strains of *Campylobacter* Isolated from Poultry

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ABSTRACT

The antibacterial effect of the lactoperoxidase/thiocyanate/hydrogen peroxide system (lactoperoxidase system) was tested against strains of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry. The effect was studied at different pH-values and temperatures in UHT-milk (control); UHT-milk containing lactoperoxidase, sodiumthiocyanate and hydrogen peroxide (lactoperoxidase system); UHT-milk containing lactoperoxidase, sodiumthiocyanate, hydrogen peroxide and sodiumpyrosulfite (inactivated lactoperoxidase system). The lactoperoxidase system had a strong bactericidal effect against *C. jejuni* and *C. coli*. The bactericidal effect was more rapid at 37°C compared to 20°C. The effect of lactoperoxidase system decreased with decreasing pH-values. The fastest reduction in viable numbers was obtained at pH 6.6 and 37°C.

Campylobacter jejuni is today recognized as a cause of acute enteritis in man *(3,13)*. A major source of infection is poultry meat *(6,12)*, but the consumption of raw milk has also been linked to several outbreaks of the disease *(7,11,15)*. Although *C. jejuni* can readily be isolated from the bovine intestinal tract, the bacteria have not frequently been isolated from raw milk. Methodology may be the most important reason for the infrequent isolation from milk. However, several investigators have suggested that the natural antimicrobial factors present in bovine milk could also be involved *(4,5)*. Beumer and coworkers *(1)* were later able to show that the lactoperoxidase/thiocyanate/hydrogen peroxide system present in bovine milk also has a bactericidal effect against strains of *C. jejuni*. They concluded that this could be one factor explaining the relatively low occurrence of *C. jejuni* in milk.

The antibacterial activity/mechanism of the lactoperoxidase system is today well-documented *(8)*. The antibacterial effect is mediated by a short-lived oxidation product of thiocyanate, hypothiocyanite ($OSCN^-$), which inhibits enzymes vital for the metabolism thereby causing an inhibition and/or killing of the organism. Hypothiocyanite can be formed *in situ* in mixtures of lactoperoxidase and thiocyanate through the addition of hydrogen peroxide or a suitable source thereof.

As mentioned previously, poultry meat is a potential risk in the spreading of the disease. The poultry carcass is often contaminated with *Campylobacter*, during the slaughter, by fecal material. In a future perspective, it could be possible to utilize the antibacterial effect of the lactoperoxidase system to eliminate or reduce this contamination during the slaughtering process. In this study we have investigated the sensitivity of strains of *Campylobacter* isolated from poultry to the lactoperoxidase system. Various times of exposure, pH, and temperatures have been tested in order to find out the optimal conditions relevant to the slaughtering process.

MATERIALS AND METHODS

Bacteria

Seven different strains of *C. jejuni* (DSl; 9/9; 2514; 2832; 5229; 5276; 2491) and two strains of *C. coli* (H708; DS3) were tested. Strains DSl and DS3 were stock cultures, while the other strains were freshly isolated from poultry. The strains were kept at -80°C in beef broth containing 10% (v/v) horse serum and 17% (v/v) glycerol.

Culture media

The strains were grown on nutrient agar CM3 (Oxoid Ltd, Basingstoke, Hants, England) supplemented with 7% (v/v) bovine blood, 0.025% (w/v) $FeSO_4$, 0.025% (w/v) $Na_2S_2O_5$, 0.025 (w/v) sodium pyruvate, and 0.1% (w/v) glucose. The plates were incubated microaerobically (8-10% CO_2; 5-7% O_2) for 16 h at 43°C. Isolated colonies were then transferred to nutrient broth (Nutrient broth No. 2, Oxoid Ltd, Basingstoke, Hants, England) containing 5% (v/v) defibrinated horse blood and incubated for 16 h at 43°C.

Exposure to the lactoperoxidase/thiocyanate/hydrogen peroxide system

The antibacterial effect was tested in 10 ml of UHT-milk (3% fat; Arla dairies, Alingsås, Sweden) fortified with 0.1% yeast extract (Oxoid Ltd, Basingstoke, Hants, England). The pH was adjusted with 10% v/v lactic acid (heat sterilized, 121°C, 15 min). The lactoperoxidase system was included through the addition of 10 µg/ml of filter sterilized (0.2 µm) lactoperoxidase (Sigma Chemical Co,

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MO, USA), 0.25mM filter sterilized sodium thiocyanate, and 0.25 mM hydrogen peroxide. To inactivate the lactoperoxidase system, 1mM filter sterilized $Na_2S_2O_5$ was added to the media.

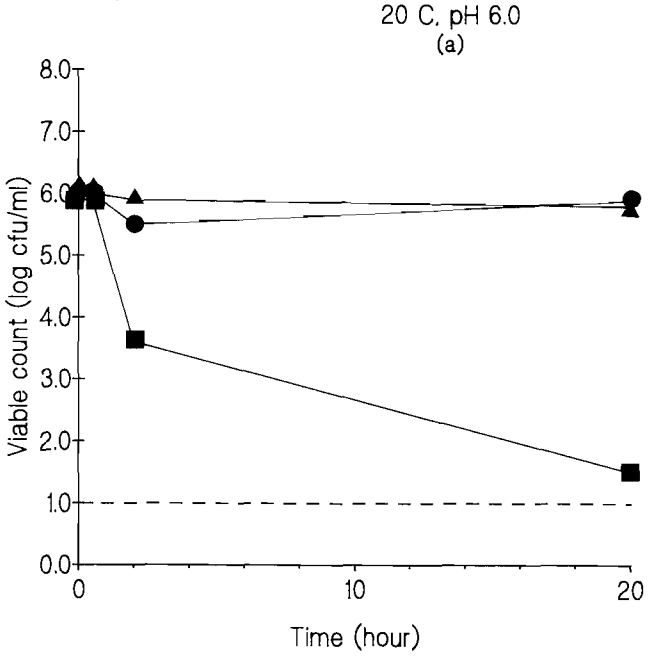
The test tubes were preincubated 10 min at the test temperature before being inoculated with 0.1 ml bacterial suspension giving about 10^6 cfu/ml. Samples were taken out at 0, 0.5, 2, and 20 h after inoculation. Between 2 and 20 h the test tubes were incubated microaerobically. Serial dilutions were made in sterile saline containing 0.1% (w/v) peptone. 10 µl of the dilutions were spread onto predried blood agar plates. In order to get a detection limit of 10 cfu/ml, also 0.1 ml was spread onto one separate plate. The plates were incubated microaerobically at 43°C for 16 to 24 h.

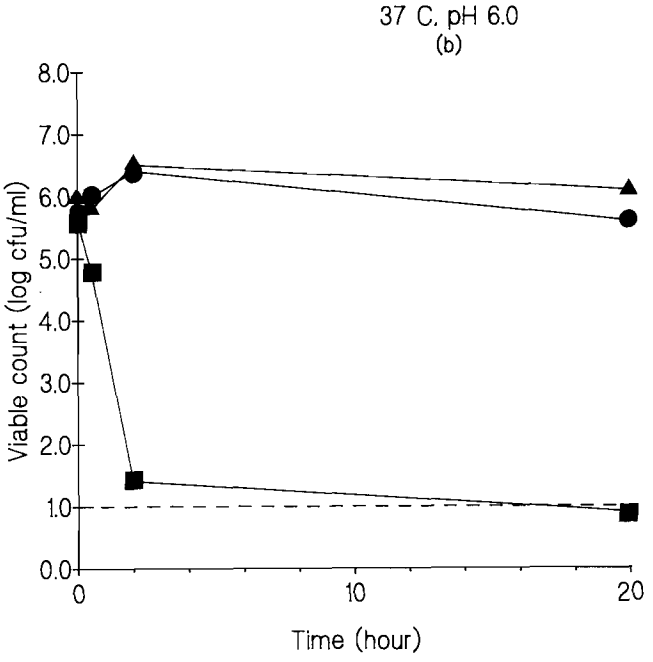
RESULTS AND DISCUSSION

The bactericidal effect of the lactoperoxidase system at different pH-values and temperatures was tested against *C. coli* DS3, *C. coli* H708, *C. jejuni* 2491, and *C. jejuni* 2832.

The bactericidal effect of the lactoperoxidase system was higher at 37°C than at 20°C (Figs. 1a and b). A similar response to the temperature is reported for *Escherichia coli (2)*. When the incubation temperature was increased to 52°C the number of *Campylobacter* was drastically reduced even in the absence of the lactoperoxidase system (Fig. 1c), however, the reduction was faster in the presence of the lactoperoxidase system.

The effect of the lactoperoxidase system decreased with decreasing pH. The highest bactericidal effect was obtained at pH 6.6 while at pH 6.0 and pH 5.5 the effect was considerably lower (Figs. 2 and 3). Previous investigations *(9)* have demonstrated the opposite, i.e. an enhanced effect of the lactoperoxidase system at lower pH-values. This may, according to Thomas *(16)*, be attributed to the fact that the major oxidation product with antibacterial effect formed by the lactoperoxidase system, $OSCN^-$, exists in an acid/base equilibrium with HOSCN (pKa = 5.3). Thus, at lower pH-values the equilibrium is towards HOSCN, which more easily penetrates the cell membranes. The unexpected results on the effect of pH in the





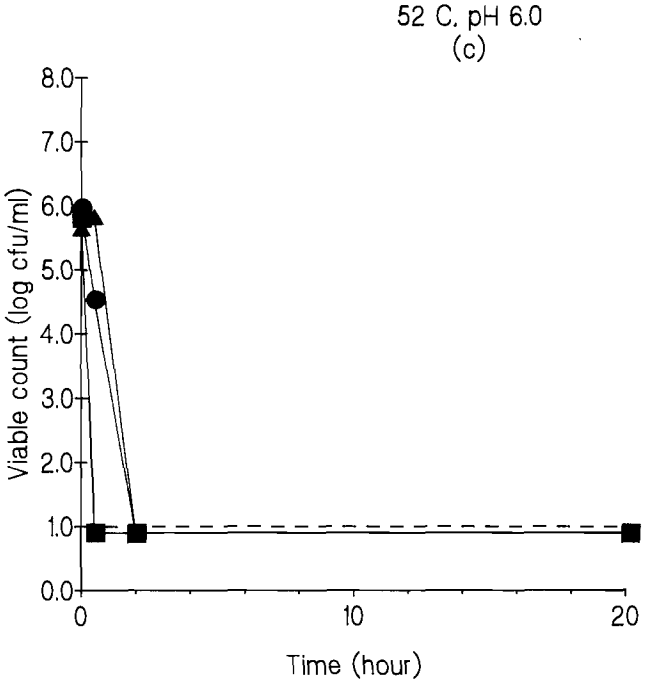
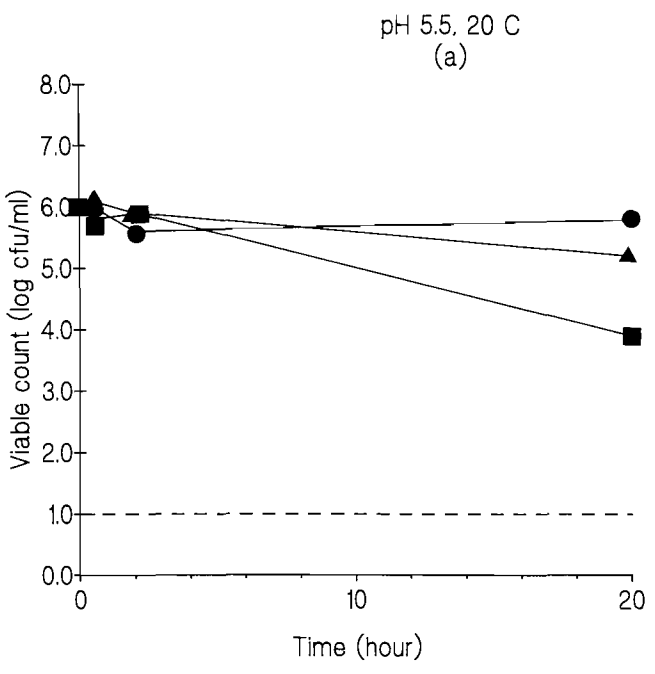
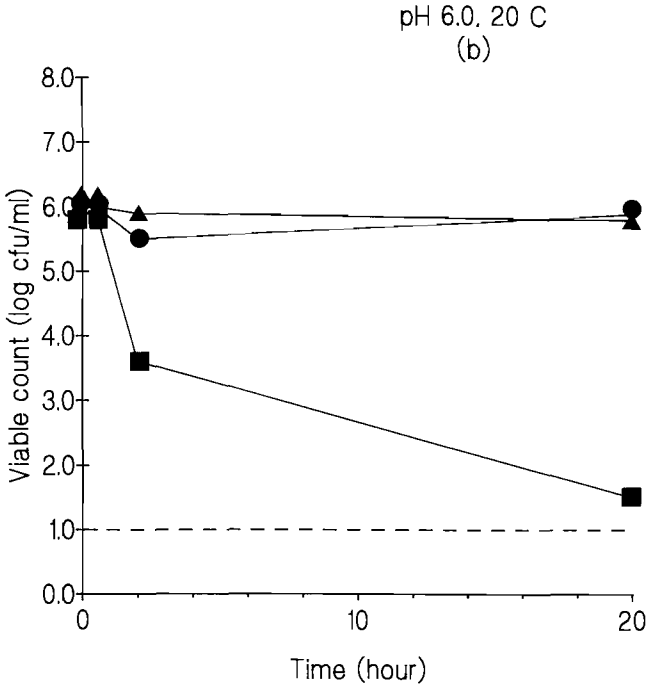


Figure 1. *Survival of* campylobacter *in (●), UHT-milk; (■), UHT-milk containing lactoperoxidase system i.e. lactoperoxidase, sodium thiocyanate, and hydrogen peroxide; (Δ),UHT-milk containing inhibited lactoperoxidase system i.e. lactoperoxidase, sodium thiocyanate, hydrogen peroxide, and soiumpyrosulfite at different temperatures (a), 20°C; (b), 37°C; (c), 52°C; and pH 6.0. Mean values of* C. coli *H708*, C. coli *DS3*, C. jejuni *2491 and* C. jejuni *2832 are shown.*





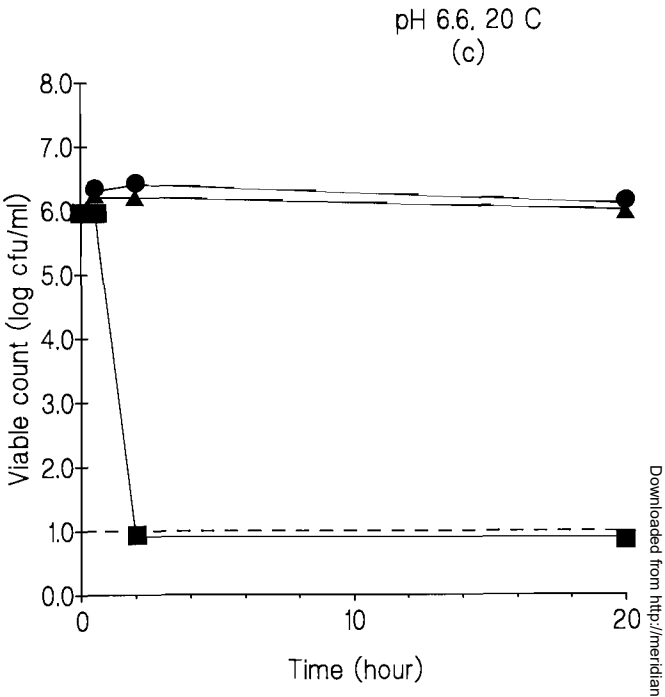


Figure 2. *Survival of* campylobacter *in (●), UHT-milk; (■), UHT-milk containing lactoperoxidase system i.e. lactoperoxidase, sodium thiocyanate, and hydrogen peroxide; (Δ),UHT-milk containing inhibited lactoperoxidase system i.e. lactoperoxidase, sodium thiocyanate, hydrogen peroxide, and soiumpyrosulfite at different pH-values (a), pH 5.5; (b), pH 6.0; (c), pH 6.6; and 20°C. Mean values of* C. coli *H708,* C. coli *DS3,* C. jejuni *2491, and* C. jejuni *2832 are shown.*

present study may be attributed to the microaerophilic nature of *Campylobacter (14)*, giving a very low tolerance towards short-lived oxidation products other than $OSCN^-$, such as O_2SCN^- and O_3SCN^- *(10)*.

Beumer et al. *(1)* demonstrated an effect against a *C. jejuni* strain of human origin by stimulating the lactoperoxidase system in milk. In the present study, the effect of the lactoperoxidase system at pH 6.6 and 37°C was tested on *C. jejuni* DS1, *C. jejuni* 9/9, *C. jejuni* 2514, *C. jejuni* 2832, *C. jejuni* 5229, *C. jejuni* 52'16, *C. jejuni* 2491, *C. coli* H708, and *C. coli* DS3. During a period of 30 min the number of viable cells was, for all tested strains, reduced from the inoculum level of 10^6 cfu/ml to a level below the detection limit (10 cfu/ml) in the presence of the lactoperoxidase system. The addition of 1 mM sodiumpyrosulfite (an inhibitor to the lactoperoxidase system) completely cancelled the bactericidal effect. Thus the reduction in viable cells could be assigned to the activity of the lactoperoxidase system.

It may be concluded that the lactoperoxidase system at pH 6.6 and 37°C has an efficacious bactericidal effect against strains of *C. jejuni* and *C. coli* isolated from poultry. The prospect that the lactoperoxidase system can be utilized to cancel/reduce contamination of *C. jejuni* from poultry during the slaughter process may be possible. How the lactoperoxidase system should be applied in a practical way to the slaughter line has to be tried out in future experiments. Such trials are presently being planned and will be reported separately.

ACKNOWLEDGMENTS

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REFERENCES

1. Beumer, R. R., A. Noomen, J. A. Marijs, and E. H. Kampelmacher. 1985. Antibacterial action of the lactoperoxidase system on *Campylobacter jejuni* in cow's milk. Neth. Milk Dairy J. 39:107-114.
2. Björck, L. 1977. Studies of the antibacterial effect of the lactoperoxidase

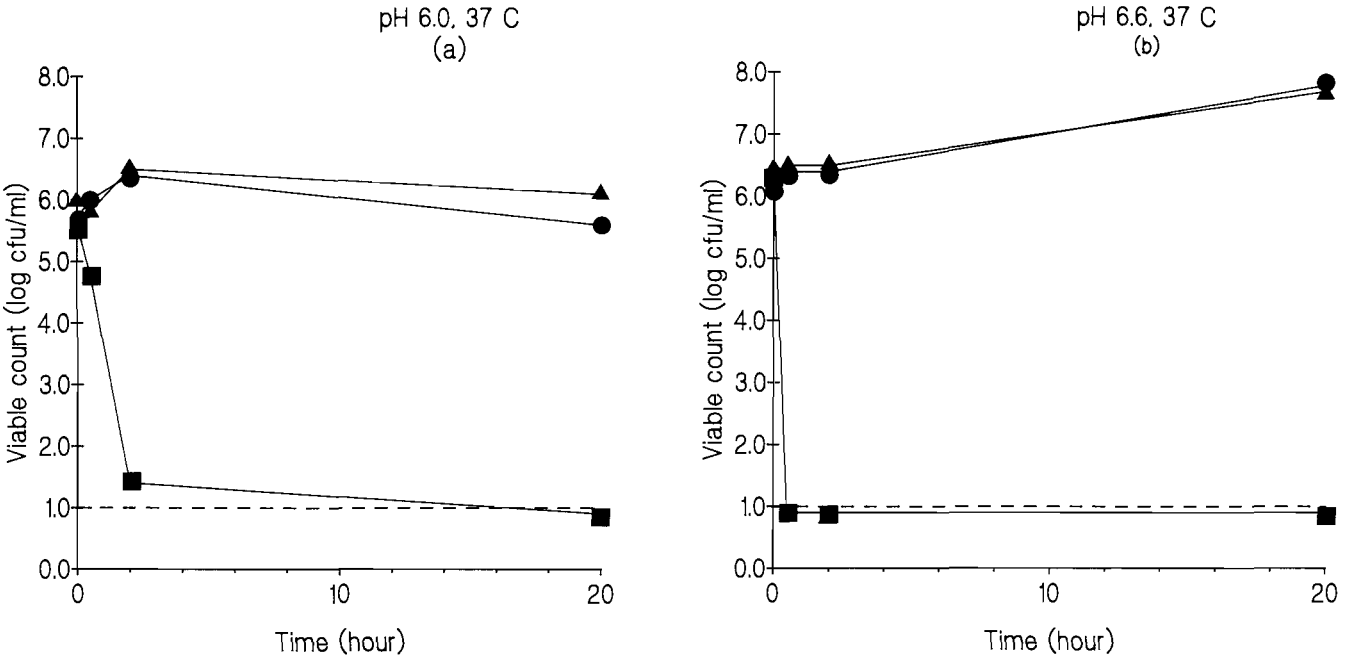

Figure 3. *Survival of* campylobacter *in (●), UHT-milk; (■), UHT-milk containing lactoperoxidase system i.e. lactoperoxidase, sodium thiocyanate, and hydrogen peroxide; (Δ),UHT-milk containing inhibited lactoperoxidase system i.e. lactoperoxidase, sodium thiocyanate, hydrogen peroxide, and soiumpyrosulfite at different pH values (a), pH 6.0; (b), pH 6.6; and 37 °C. Mean values of* C. coli *H708,* C. coli *DS3,* C. jejuni *2491, and* C. jejuni *2832 are shown.*

system on some gram-negative bacteria. Thesis Swedish University of Agricultural Sciences, Uppsala, Sweden.
3. Blaser, M. J., F. M. LaForce, and N. A. Wilson. 1980. Reservoirs for human Campylobacteriosis. J. Infect. Dis. 141:665-669.
4. de Boer, E. B., J. Hartog, and G. H. A. Borst. 1984. Milk as a source of *Campylobacter jejuni*. Neth. Milk Dairy J. 38:183-194.
5. Doyle, M. P., and D. J. Roman. 1982. Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. Appl. Environ. Microbiol. 44:1154-1158.
6. Engvall, A., Bergqvist, K. Sandstedt, and M. -L. Danielsson-Tham. 1986. Colonization of broilers with *Campylobacter* in conventional broiler-chicken flocks. Acta Vet. Scand. 27:540-547.
7. Porter, I. A., and T. M. S. Reid. 1980. A milk-borne outbreak of *Campylobacter* infection. J. Hyg. Cambridge 84:415-419.
8. Pruitt, K., and J. Tenovuo (eds.). 1985. The lactoperoxidase system; Chemistry and biological significance. Marcel Dekker Inc., New York.
9. Reiter, B., Valerie M. E. Marshall, L. Björck, and C. -G. Rosén. 1976. Nonspecific bactericidal activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system of milk against *Escherichia coli* and some Gram-negative pathogens. Infect. Immun. 13:800-807.
10. Reiter, B., and G. Härnulv. 1984. Lactoperoxidase antibacterial system: Natural occurrence, biological functions and practical applications. J. Food Prot. 47:724-732.
11. Robinsson, D. A., and D. M. Jones. 1981. Milk-borne *Campylobacter* infection. Br. Med. J. 282:1374-1376.
12. Shanker, S., J. A. Rosenfield, G. R. Davey, and T. C. Sorell. 1982. *Campylobacter jejuni*: Incidence in processed broilers and biotype distribution in human and broiler isolates. Appl. Environ. Microbiol. 43:1219-1220.
13. Skirrow, M. B. 1982. *Campylobacter* enteritis -- the first five years. J. Hyg. 89:175-184.
14. Smibert, R. M. 1984. *Campylobacter*. pp.111-117. *In* N. R. Krieg (ed.), Bergey's Manual of Systematic Bacteriology. Williams & Wilkins, Baltimore/London.
15. Taylor, D. N., B. W. Porter, and C. A. Williams. 1982. *Campylobacter* enteritis: A large outbreak traced to commercial milk. West. J. Med. 147:365-369.
16. Thomas, E. L. 1981. Lactoperoxidase-catalyzed oxidation of thiocyanate: equilibria between oxidized forms of thiocyanate. Biochem. 20:3273-3280.