EFFECTS OF NITROETHANE AND 2-NITROPROPANOL AGAINST CAMPYLOBACTER JEJUNI

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Abstract Campylobacter jejuni is an important foodborne pathogen that colonizes the gut of swine. In this study, the effects of nitroethane and 2-nitropropanol (0, 10 and 20 mM) on growth of C. jejuni were tested during culture in Bolton broth adjusted to pH of 5.6, 7.0 or 8.2. Viable cell counts of samples taken at intervals during incubation revealed main effects (P<0.0001) of nitroethane or 2-nitropropanol on mean specific growth rates thus demonstrating that these were inhibitory to C. jejuni. By 48 h of incubation, C. jejuni concentrations had increased by 1.9 log₁₀ CFU/ml or higher in cultures containing no added nitrocompound. In contrast, C. jejuni concentrations had decreased by >1.8 and >5.4 log₁₀ CFU/ml after 48 h incubation with 20 mM nitroethane or 2-nitropropanol, respectively. A marked effect of pH on the inhibitory activity of both compounds was observed, with greater activity observed at higher pH.

Introduction Approximately 76 million cases of human foodborne disease are estimated to occur in the United States each year (Mead et al, 1999) at a cost of more than $7 billion annually (ERS/USDA, 2001). Nearly 2.5 million of these infections were attributed to Campylobacter jejuni (Mead et al, 2000). Campylobacter species have been reported to be the most common causes of acute bacterial diarrhea worldwide (Linton et al, 1997; Nachamkin et al, 1998) and are associated with immune-mediated neuropathies such as Guillain-Barré Syndrome and the Miller Fisher syndrome (Ang et al, 2001). Campylobacter species are ubiquitous colonizers of the gastrointestinal tracts of domestic and feral animals (Jones, 2001), with prevalence in swine reported at more than 80% (Pearce et al, 2003). Consequently, strategies are sought to reduce concentrations of these bacteria in swine before they arrive for processing, especially since quantitative risk assessments indicate that such interventions may significantly reduce human exposures to the pathogens (Vugia et al, 2003).

Recent studies have shown that 2-nitropropanol exhibits broad spectrum antimicrobial activity against Salmonella serovar Typhimurium, Escherichia coli 0157:H7 and Enterococcus faecalis in vitro (Jung et al, 2004a), against Salmonella Typhimurium in broilers (Jung et al, 2004b) and against ruminal methane producing activity (Anderson et al, 2003). Likewise, nitroethane has been reported to reduce gut concentrations of Salmonella and Campylobacter in pigs (Jung et al, 2003). Reductions in animal studies have been inconsistent, however, thus suggesting that certain conditions may limit the activity of these compounds (unpublished). The present study was conducted to measure the effects of pH on the bactericidal activity of nitroethane and 2-nitropropanol against C. jejuni in vitro.

Materials and Methods Campylobacter jejuni (CC326) was obtained during sampling of a California dairy (Harvey et al, 2004). Strain 326 was inoculated and grown in 10 ml Bolton Broth supplemented with 0, 10 or 20 mM nitroethane or 2-nitropropanol and adjusted to pH 5.6, 7.0 or 8.2. Tubes were incubated in triplicate at 42°C under microaerophilic conditions (10% CO₂, 5% O₂ and 85% N₂). Samples collected at 0, 6, 24 and 48 hour times during incubation were serially diluted, plated to Campy Cephex Agar and incubated similarly to quantify viable colonies. Test for effects of nitrocompound, pH and nitrocompound x treatment interaction were conducted by general analysis of variance (Statistix®8 Analytical Software, Tallahassee, FL) using mean specific growth rates determined after 24 h incubation.

Results/Discussion Recent laboratory tests with the electro-negative nitrocompounds, 2-nitropropanol, 2-nitroethanol or nitroethane had shown inclusion of these compounds into in vitro incubations pig fecal flora markedly enhanced the bactericidal activity of chlorate against Salmonella Typhimurium (Anderson et al, 2004). The nitrocompounds by themselves exhibited bactericidal
activity against *Salmonella* and this activity was more persistent than that of chlorate alone (Anderson *et al.*, 2004). Whereas chlorate is consumed by nitrate reductase activity (Gennis and Stewart, 1996), there is no evidence that the nitrocompounds are reduced by nitrate reductase which may explain why their activity is more persistent than chlorate. In the present study, the inhibitory activity of nitroethane and 2-nitropropanol on growth *C. jejuni* during incubation in Bolton broth is clearly evident (Figures 1 and 2, respectively). In the case of 2-nitropropanol, and with higher concentrations of nitroethane, the activity appears to be bactericidal as recovery of *C. jejuni* on Campy Cephex Agar plates was markedly reduced. We can not rule out, however, the possibility that the non-recovered cells had been induced into a viable but nonculturable state.

Results presented here show that 2-nitropropanol was more effective against *C. jejuni* than nitroethane at all pH ranges although the activity of both compounds was greater at pH 8.2, possibly because the nitrocompounds, possessing labile protons next to the nitro group, would be expected to be more reactive at a higher pH. These findings have practical implications considering that ileal, cecal and colonic contents of weaned pigs are typically pH 7.0 or less (Harvey *et al.*, 2001; Mathew *et al.*, 1993; Prohászka and Lukács, 1984) although the pH of cecal contents in fasted pigs was more alkaline at pH 7.5 (Harvey *et al.*, 2001). Research is underway with these and other nitrocompounds to determine if these exhibit inhibitory activity against other foodborne pathogens and to better understand the limits of their activity.

**Conclusion** Results presented here confirm the bactericidal activity of nitroethane and 2-nitropropanol against *C. jejuni* *in vitro*. Although these nitrocompounds have shown significant inhibitory effect, their mechanism of action is unknown. Results from this study demonstrate that nitro-inhibition of *C. jejuni* growth is pH and concentration dependent.

**References**


Harvey, R.B., Anderson, R.C., Young, C.R., Swindle, M.M., Genovese, K.J., Hurme, M.E., Droleskey, R.E., Farrington, L.A., Ziprin, R.L., Nisbet, D.J., 2001. Effects of feed withdrawal and transport on cecal environment and *Campylobacter* concentrations in a swine surgical model. *J Figure 1. Effect of nitroethane on *Campylobacter jejuni* during growth in Bolton broth adjusted to pH 5.6 (A), 7.0 (B) or 8.2 (C). Values are the mean from 3 replicates, SD were less than 0.15 log_{10} CFU/ml unless indicated otherwise. Mean specific growth rates determined after 24 h are presented.


Figure 2. Effect of 2-nitropropanol on Campylobacter jejuni during growth in Bolton broth adjusted to pH 5.6 (A), 7.0 (B) or 8.2 (C). Values are the mean from 3 replicates, SD were less than 0.15 log_{10} CFU/ml unless indicated otherwise. Mean specific growth rates determined after 24 h are presented.