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Escherichia coli O157 and Non-O157 Isolates Are More Susceptible to L-Lactate than to D-Lactate

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The antimicrobial effect of L-lactate was much greater than that of D-lactate over a range of concentrations for *Escherichia coli* O157 and non-O157 strains. Despite this, the intracellular pHs and membrane potentials of L-lactate- and D-lactate-treated cells were similar, suggesting that these factors are not involved in the antimicrobial action of L-lactate.

Escherichia coli O157 is a major cause for concern in the field of public health. Infections caused by this bacterium, though infrequent, are associated with high levels of morbidity and mortality and may have led to reduced public confidence in food safety, particularly for red meat products. There is growing interest in both how *E. coli* O157 enters the food chain and practical measures of prevention.

Organic acids have been used traditionally in abattoirs and the animal feed, food, and pharmaceutical industries to control pathogens (4). Recent evidence has suggested that these acids may be effective in controlling the proliferation of *E. coli* O157 (7). One of the most effective acids against this organism in vitro is lactate, and its effectiveness in combination with its wide availability, low cost, and generally "recognized-as-safe" status makes lactate a promising candidate as a control measure for *E. coli* O157 in farm and slaughterhouse environments. Investigations into the antimicrobial effect of lactate have previously focused on either L-lactate or, more frequently, the commercially available DL-lactate mixture which contains the isomers in variable proportions. A preliminary study at the Rowett Research Institute (11) has suggested that *E. coli* strains from pigs may be more susceptible to L-lactate than to D-lactate. We decided to extend this study to compare the relative contributions of the stereoisomers to the antimicrobial effect of lactate on various *E. coli* O157 and non-O157 isolates.

The *E. coli* strains used in this study are shown in Table1. Stationary-phase cells were prepared as previously described (10) to give a population of approximately 10^9 CFU ml^{-1}. Cultures were treated with L-lactate (Sigma, Poole, United Kingdom) or D-lactate (Sigma) to produce a final pH of 3.8, and the viability was then determined (10). Specific death rates (SDR) were calculated by plotting the results of viability studies over time semilogarithmically and determining the negative value of the slope. The reduction in viable count could then be expressed as $\log_{10}$ CFU per milliliter per hour. All viability studies were performed in duplicate, and the results were compared using analysis of variance.

The effects of the individual stereoisomers of lactate on *E. coli* NCTC 12900 and F318 were examined. L-Lactate had a much greater antimicrobial effect than did D-lactate, reflecting previously published observations (11). The efficacies of both isomers were dose dependent, and a greater concentration of D-lactate than of L-lactate was required to obtain similar reductions in viability. The isomers and their concentrations in ascending order of efficacy ($P < 0.05$) were as follows: 100 mM D-lactate < 50 mM L-lactate < 150 mM D-lactate < 100 mM L-lactate (Fig. 1). The effects of treatments consisting of 200 mM D-lactate, 150 mM L-lactate, and 200 mM L-lactate did not differ from each other statistically but were greater ($P < 0.05$) than those of the other treatments.

The viability of strain NCTC 12900 in various concentrations of D-lactate or L-lactate was determined. Stationary-phase cultures were challenged with various proportions of L-lactate and D-lactate such that the total concentration was 100 mM. L-Lactate at a concentration of 100 mM exerted a greater antimicrobial effect ($P < 0.01$) than 100 mM D-lactate for NCTC 12900 (Fig. 2A) and F318 (Fig. 2B). Increasing the proportion of the L isomer over that of the D isomer heightened the antimicrobial efficacy in a dose-dependent manner for both strains.

A range of O157 and non-O157 *E. coli* isolates were assayed to determine whether the greater efficacy of L-lactate than of D-lactate is widespread in *E. coli* strains. Stationary-phase cells were incubated in 100 mM L-lactate or D-lactate for 3 h. The isolates were much less sensitive ($P < 0.00001$) to D-lactate than to L-lactate, suggesting that this effect is prevalent among *E. coli* strains (Table 1). These results may have implications for improving the efficacy of lactic acid decontaminant preparations and probiotic lactic acid bacteria. The abilities of the various strains to survive lactate challenge were highly variable. When the *E. coli* isolates were grouped as O157 or non-O157 strains, there was no statistical difference between the groups in their sensitivities to D-lactate. However, the non-O157 strains were more susceptible to L-lactate than were the O157 strains. Previous studies comparing the levels of acid tolerance of *E. coli* strains have suggested that either *E. coli* O157 strains are more acid tolerant than non-O157 *E. coli* strains, or there is no difference between the two groups (2).

Ethanol has been previously shown to enhance the killing of *E. coli* O157 strains by lactate (7). We examined the effect of ethanol on D- and L-lactate-mediated viability by using stationary-phase cultures treated with 5% (vol/vol) ethanol and a 100 mM concentration of the organic acid (Table 2). The SDR of

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TABLE 1. Effect of lactate on the viability of various O157 and non-O157 *E. coli* strains

Strain	Origin	Serotype	Decrease in viability[a]	
			D-Lactate	L-Lactate
NCTC 12900	Human	O157:H7 (VT−)[b]	−0.08	10.2
NCTC 13126	Human	O157:H7 (VT−)	0.92	8.52
NCTC 12079	Human	O157:H7	7.6	6.40
AUIO-5[c]	Cattle feces	O157:H7	0.42	4.66
AUIO-7[c]	Raw milk	O157:H7	0.08	4.77
AUIO-13[c]	Minced beef	O157:H7	3.53	6.00
AUIO-309[c]	Cheese	O157:H7	0.17	4.76
AUIO-ND[c]	Sheep feces	O157:H7	1.42	4.49
F318	Sheep rumen	O162	1.25	10.4
F38	Sheep rumen	O rough	4.08	8.83
EC17	Pig	O106:NM	−0.25	10.6
EC30	Bison	O113:H21	6.83	16.1
EC33	Sheep	O7:H21	2.00	15.4
EC45	Pig	ON:HM	1.58	13.2
EC47	Sheep	ON:H18	−2.08	5.75
EC67	Goat	O4:H43	0.08	9.42

[a] Decrease in viable cell numbers, expressed in CFU (10^8) ml^{-1}, following 3 h of incubation in 100 mM lactate at pH 3.8. Negative values indicate growth. Results are the average of two repeat experiments, and the average standard deviation was 1.54×10^8 CFU ml^{-1}.

[b] VT−, verocytotoxin negative.

[c] Kindly supplied by Ian Ogden, Department of Medical Microbiology, University of Aberdeen, Aberdeen Royal Hospitals Trust, Foresterhill, Aberdeen, United Kingdom.

NCTC 12900 treated with ethanol and D-lactate (1.36 $\log_{10}$ CFU ml^{-1} h^{-1}) was much greater ($P < 0.05$) than that of NCTC 12900 treated with D-lactate alone (0.16 $\log_{10}$ CFU ml^{-1} h^{-1}). This result concurs with previous data for DL-lactate (2). The SDR were similar for NCTC 12900 treated with L-lactate both with and without ethanol (2.00 and 2.12 $\log_{10}$ CFU ml^{-1} h^{-1}, respectively). Ethanol increased the SDR of D-lactate and L-lactate-treated F318 cells. Perhaps the ability of ethanol to perturb membranes or the alteration in fatty acid composition associated with alcohols increased the uptake of the undissociated acid (8).

The effect of temperature on the antimicrobial effect of lactate was investigated. Stationary-phase cultures of NCTC 12900 and F318 were incubated at 20°C in the presence of 100 mM D-lactate or L-lactate. The SDR of cells treated at 20°C were lower than those of the lactate-treated isolates at 37°C (Table 2). This finding concurs with previously reported data showing that O157 strains became more susceptible to 1.5% lactic acid with increasing temperature (14). Acid dissociation

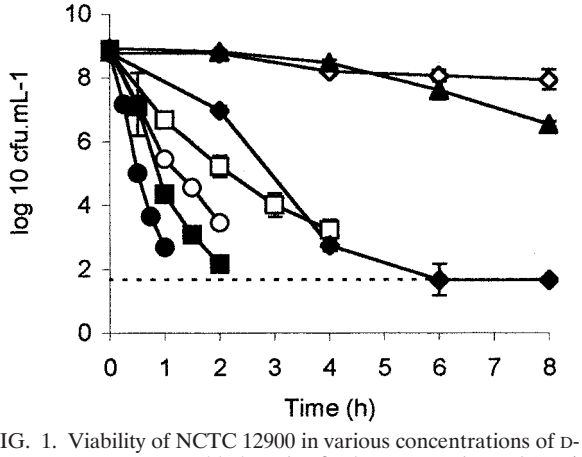

FIG. 1. Viability of NCTC 12900 in various concentrations of D- or L-lactate. D-Lactate was added to give final concentrations of 100 (◇), 150 (□), and 200 (○) mM. L-Lactate was added to give final concentrations of 50 (▲), 100 (◆), 150 (■), and 200 (●) mM. A dashed line represents the limit of detection (1.67 $\log_{10}$ CFU ml^{-1}).

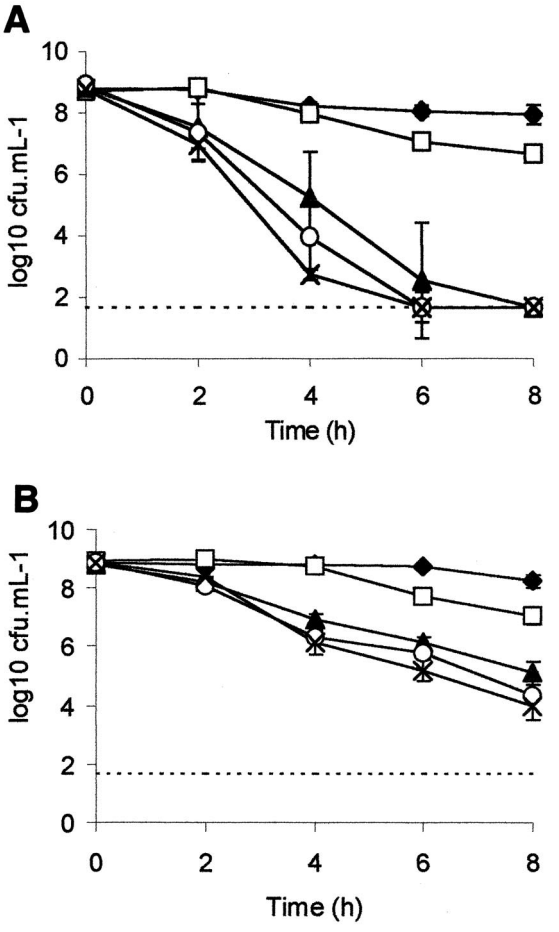

FIG. 2. Viability of NCTC 12900 (A) and F318 (B) treated with various combinations of D- and L-lactate. Acids were added to give a final combined concentration of 100 mM and consisted of 100 mM D-lactate (◆), 75 mM D-lactate plus 25 mM L-lactate (□), 50 mM D-lactate plus 50 mM L-lactate (▲), 25 mM D-lactate plus 75 mM L-lactate (O), and 100 mM L-lactate (×). A dashed line represents the limit of detection (1.67 $\log_{10}$ CFU ml^{-1}).

TABLE 2. SDR following various treatments with 100 mM lactate

Treatment[b]	SDR ($\log_{10}$ CFU ml^{-1} h^{-1})[a]	
	NTCC 12900	F318
D-Lactate	0.16ABC	0.24A
L-Lactate	2.12B	0.71
D-Lactate + ethanol	1.36C	0.79D
L-Lactate + ethanol	2.00	2.62D
D-Lactate, 20°C	0.04	0.04
L-Lactate, 20°C	0.57	0.07

[a] SDR were calculated as the reduction in the viable count per hour and are the average of two repeat experiments. SDR followed by the same letters differ statistically.

[b] The final pH of the cultures was 3.8, and the concentration of ethanol was 5%. Unless otherwise stated, the temperature was 37°C.

TABLE 3. Effect of lactate on the proton motive force

Strain	Acid	Temp (°C)	pH_o[a]	pH_i[b]	ΔpH[c]	$Z\Delta pH$[d]	$\Delta\Psi$[e]	Δp[f]
NCTC 12900	L-Lactate	37	3.76	4.90	1.14	70.68	−42.92	−113.60
	D-Lactate	37	3.84	4.86	1.02	63.05	−37.54	−100.63
F318	L-Lactate	37	3.81	4.34	0.53	32.65	−20.35	−53.00
	D-Lactate	37	3.79	4.30	0.51	31.62	−25.01	−56.63

[a] pH_o, external pH in pH units.
[b] pH_i values (pH units) are the average of three to five repeat experiments.
[c] pH gradient ($pH_i - pH_o$) expressed in pH units.
[d] pH gradient expressed in millivolts, where Z is 62 at 37°C and 58 at 20°C.
[e] Membrane potentials, expressed in millivolts, are the average of three to five repeat experiments.
[f] Proton motive force expressed in millivolts ($\Delta\Psi - Z\Delta pH$).

constants are inversely affected by temperature (4). The steady-state intracellular lactate anion concentration that accumulated at 37°C would therefore be greater than that at 20°C, assuming that lactate is able to freely permeate the cytoplasmic membrane. This may explain the reduced susceptibility of *E. coli* strains to lactate at 20°C. Another possible explanation is that alterations in the fatty acid content of *E. coli* membranes occur at lower temperatures (8).

The antimicrobial mode of action of organic acids has not been satisfactorily explained (4). Traditionally, only undissociated acid was thought to freely permeate the membrane, where it released toxic acid anions and protons intracellularly according to the intracellular pH (pH_i), the protons causing acidification of the cytoplasm and dissipation of the transmembrane proton potential (6). However, this rationale has been dismissed as too simplistic (4), and other mechanisms have been proposed. Cherrington et al. (5) showed that bacteriostatic concentrations of propionic and formic acids interfered with *E. coli* macromolecular synthesis and it has been proposed that sorbic acid acts as a membrane-active compound for yeasts (13). Jordan et al. (7) showed that lactate caused a reduction in the pH_i and suggested that the proton gradient (ΔpH) had collapsed. We decided to investigate the components of the proton motive force with respect to D-lactate and L-lactate.

The pH_i, the ΔpH, and the membrane potential ($\Delta\Psi$) were determined by a centrifugation method described previously (9). Stationary-phase cells were incubated in 100 mM D- or L-lactate for 10 min. Measurements were performed three to five times. As shown in Table 3, the ΔpH values of cells treated with D-lactate or L-lactate were similar (1.02 and 1.14, respectively), as were the $\Delta\Psi$ values (−37.54 and −42.92 mV, respectively). Similar trends were observed for F318 cells (Table 3). Although the $\Delta\Psi$ measurements were relatively low, they were comparable to previous results for nongrowing *E. coli* in anaerobic environments (1). D-Lactate, which has little antimicrobial effect at 100 mM, caused a reduction in pH_i similar to that of the bactericidal agent L-lactate. This suggests that the antimicrobial action of L-lactate is not due to the collapse of the pH_i, as previously suggested (7). The reduction in pH_i is probably due to a tolerance mechanism that prevents the accumulation of large amounts of acid anions, which was suggested previously (12). As neither the ΔpH nor the $\Delta\Psi$ has been abolished, it is unlikely that the toxicity of lactate is due to uncoupling. Given that as D-lactate is the major fermentative product of *E. coli* at low pH under anaerobic conditions (3), it is possible that this organism has developed methods of effectively dealing with high intracellular concentrations of D-lactate, such as efflux mechanisms or conversion to nonacidic end products.

It has been suggested that direct comparisons of different organic acids with respect to their antimicrobial activities are difficult because of the variation in physical characteristics (4). Studies comparing the antimicrobial effects of the isomers of lactate may provide a useful tool in elucidating the mechanism of action of this acid. As the isomers of lactate have the same pK_as and similar structures, it is likely that they will share a similarity in nonspecific interactions but will differ with respect to specific interactions such as enzymatic reactions.

In conclusion, L-lactate has a much greater antimicrobial effect than D-lactate for a wide range of *E. coli* O157 and non-O157 isolates. This finding may have implications for the use of lactate as an antimicrobial agent and the use of lactic acid bacteria as probiotics. There was no difference between D-lactate- and L-lactate-treated cells with respect to the transmembrane pH gradient, suggesting that the antimicrobial mode of action of L-lactate does not involve abolition of the pH_i. This study also highlights the potential use of the isomers of lactate as a tool for elucidating the mechanism of action of lactate.

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REFERENCES

1. **Axe, D. D., and J. E. Bailey.** 1995. Transport of lactate and acetate through the energized cytoplasmic membrane of *Escherichia coli.* Biotechnol. Bioeng. **47:**8–19.
2. **Booth, I. R., F. M. Thomson-Carter, P. Carter, S. Jordan, S. Park, L. Malcolm, and J. Glover.** 1999. Acid tolerance of *Escherichia coli*—the sting in the tail?, p. 27–38. In C. S. Stewart and H. J. Flint (ed.), *Escherichia coli* O157 in farm animals. CAB International, Wallingford, United Kingdom.
3. **Bunch, P. K., F. Mat-Jan, N. Lee, and D. P. Clark.** 1997. The *idhA* gene encoding the fermentative lactate dehydrogenase of *Escherichia coli*. Microbiology (Reading) **143:**187–195.
4. **Cherrington, C. A., M. Hinton, G. S. Mead, and I. Chopra.** 1991. Organic acids: chemistry, antibacterial activity and practical applications. Adv. Microb. Physiol. **32:**87–108.
5. **Cherrington, C. A., M. Hinton, and I. Chopra.** 1990. Effect of short-chain organic acids on macromolecular synthesis in *Escherichia coli*. J. Appl. Bacteriol. **68:**69–74.
6. **Eklund, T.** 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J. Appl. Bacteriol. **69:**814–821.
7. **Jordan, S. L., J. Glover, L. Malcolm, F. M. Thomson-Carter, I. R. Booth, and S. F. Park.** 1999. Augmentation of killing of *Escherichia coli* O157 by combinations of lactate, ethanol, and low-pH conditions. Appl. Environ. Microbiol. **65:**1308–1311.
8. **Kadner, R. J.** 1996. Cytoplasmic membrane, p 58–87. *In* F. C. Neidhardt et al. (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed.; vol. 1. American Society for Microbiology, Washington, D.C.
9. **Kroll, R. G., and I. R. Booth.** 1981. The role of potassium transport in the generation of a pH gradient in *Escherichia coli*. Biochem. J. **198:**691–698.
10. **McWilliam Leitch, E. C., and C. S. Stewart.** Susceptibility of *Escherichia coli* O157 and non-O157 isolates to lactate. Lett. Appl. Microbiol., in press.
11. **Probiotic Consortium.** 1993. Efficacy and mode of action of probiotics, vol. 1. Non-ruminants, p. 93–95. Rowett Research Institute, Aberdeen, United Kingdom.
12. **Russell, J. B.** 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. J. Appl. Bacteriol. **73:**363–370.
13. **Stratford, M., and P. A. Anslow.** 1998. Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid preservative.' Lett. Appl. Microbiol. **27:**203–206.
14. **Venkitanaryanan, K. S., T. Zhao, and M. P. Doyle.** 1999. Inactivation of *Escherichia coli* O157:H7 by combinations of GRAS chemicals and temperature. Food Microbiol. **16:**75–82.