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Survival of acid-adapted *Escherichia coli O157:H7* and not-adapted *E. coli* on beef treated with 2% or 5% lactic acid☆

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ABSTRACT

Decontamination of beef by spraying with solutions of lactic acid is common practice in North America and is to be permitted in the EU. Validation of each such treatment is necessary for HACCP purposes. The utility of validation by reference to reduction in numbers of *Escherichia coli* naturally present on beef is questioned. This is because reductions of *E. coli* generally might be greater than reductions of the main target organism, *E. coli* O157:H7, which can be more acid tolerant. To investigate the effects of lactic acid sprays on the two types of *E. coli* on beef surfaces, slices of beef with cut muscle, fat or membrane surfaces were prepared. The surfaces were inoculated with a five strain cocktail of acid-adapted *E. coli* O157:H7 or with a not-acid-adapted strain of *E. coli* at number of 5, 1 or −1 log cfu/cm^2. Inoculated slices were not treated or were sprayed with water or 2% or 5% lactic acid at 0.5 ml/cm^2. For each slice of lactic acid-treated meat the numbers of *E. coli* or *E. coli* O157:H7 recovered on agars that allowed resuscitation of injured cells or did not allow resuscitation were similar. The differences in the log mean numbers (log *A*) and in the log total numbers (*N*) of bacteria recovered from slices that were not treated or treated with acid were calculated. Differences in log *A* and *N* for bacteria recovered from slices treated with water or acid were calculated also. Means of the differences indicated that each acid treatment gave similar reductions in the numbers of *E. coli* or *E. coli* O157:H7. However, reductions were somewhat greater with 5% than with 2% lactic acid. The findings suggest that the *E. coli* or *E. coli* O157:H7 that survived acid treatments of meat surfaces were protected from exposure to injurious concentrations of undissociated acid. Consequently, strains of *E. coli* that are or are not relatively tolerant of acid conditions will be inactivated to similar extents by solutions of lactic acid applied to beef surfaces.

1. Introduction

Illness caused by verotoxigenic *Escherichia coli* O157:H7 acquired from beef is a continuing cause of concern (Dodd & Powell, 2009; Pennington, 2010). To address this and other microbiological concerns beef packing plants in North America are applying various decontaminating treatments to beef (Koohmaraie et al., 2005). These include spraying carcass, cuts and trimmings with solutions of lactic acid. Decontamination of beef with lactic acid solutions will soon be permitted in the EU and elsewhere (European Food Safety Authority, 2011; The Poultry Site, 2012). Decontaminating treatments are usually Critical Control Points (CCPs) in the Hazard Analysis Critical Control Point (HACCP) systems of beef packing plants. Validation of the effects of such treatments in commercial processes is therefore necessary (Thippareddi, Boyle, & Burson, 2005). Determination of the effects of decontaminating treatments on the *E. coli* naturally present on beef is usually a practicable method of validation. However, the relevance of such findings is open to question if the pathogenic strains of major concern, such as *E. coli* O157:H7, are more resistant to a treatment than *E. coli* generally.

The effects on bacteria inoculated onto beef of solutions of lactic acid at concentrations up to 5% have been extensively studied (Loretz, Stephan, & Zweifel, 2011). In many of these studies a cocktail of *E. coli* O157:H7 strains was used as an inoculum. This has commonly been seen as necessary because, among *E. coli* in general, the acid tolerance of *E. coli* O157:H7 is relatively high (Willshaw, Thomas, & Smith, 2000). Findings for the effects of lactic acid solutions on inocula of non-O157 *E. coli* or *E. coli* naturally present on beef may then not reflect the effects of treatments on *E. coli* O157:H7 (Echeverry et al., 2010). Thus, in studies in which beef inoculated with acid-adapted

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and not adapted *E. coli* O157:H7 were treated with solutions of acetic or lactic acid, it was found that the reduction in numbers of acid-adapted cells were less than those of not-adapted cells (Berry & Cutter, 2000; Stopforth et al., 2004; Stopforth, Skandamis, Geornaras, & Sofos, 2007). However, in a recent study, cut muscle, fat and membrane covered beef surfaces were inoculated with *E. coli* and sprayed with various volume of 5% lactic acid (Youssef, Yang, Badoni, & Gill, 2012). The findings indicated that, irrespective of the numbers of inoculated bacteria, some were protected from exposure to the solution. If that is so, then differences in acid tolerance would not affect the survival of different strains of *E. coli* on beef surfaces sprayed with solutions of lactic acid. Therefore, further studies of treatment of beef surfaces with lactic acid solutions were performed using both the strain of *E. coli* used in the previous study and a cocktail of five strains of *E. coli* O157:H7.

For this study, the strains of *E. coli* O157:H7 were adapted to acid conditions. The other strain of *E. coli* was not so adapted. The acid sensitivities of the single strain and the cocktail were determined to ensure that their sensitivities were indeed different. The bacteria that survived acid treatments were recovered on agars that inhibited recovery of injured cells and also on agars that permitted resuscitation. This was done to determine if surviving cells could include large fractions of injured cells. Injury would indicate exposure to sub-lethal acid concentrations that acid tolerant cells could be expected to survive better than less tolerant cells. Also, if acid tolerance is important for survival of lactic acid treatments the survival benefit of acid tolerance could be expected to be greater with lower than with the higher concentration of lactic acid. Therefore, inoculated meat was treated with both 2% and 5% lactic acid solutions.

2. Materials and methods

2.1. Preparation of inocula

The organisms used in the study were a wild type strain of *E. coli* from a beef packing plant that had been used in a previous study; and five strains of shiga toxin-negative *E. coli* O157:H7 from the culture collection of the Food Science Department of the University of Manitoba. The five strains, designated 0304, 0627, 0648, 1840 and 3581, were kindly provided by Professor R.A. Holley. The organisms were maintained on slants of nutrient agar (Difco; Becton–Dickinson, Sparks, MD, USA), and were cultivated overnight, at 35 °C, in half strength Brain Heart Infusion (BHI, Difco) to obtain cultures from which to prepare inocula.

Inocula of *E. coli* were prepared by transferring 0.1 ml of an overnight culture into 10 ml of half strength BHI. The new culture was grown overnight at 35 °C to the stationary phase. Cultures were diluted with 0.1% (wt/vol) peptone water (Difco) to obtain suspensions containing *E. coli* at the numbers required for inoculating lactic acid solutions or meat surfaces.

Inocula of *E. coli* O157:H7 strains were prepared by first growing each strain separately, to the stationary phase, in half strength BHI supplemented with glucose at 10 g/L. Supplementing BHI with glucose results in acidification of the growth medium, and adaptation of the cultured cells to acid conditions (Sharma, Alder, Harrison, & Beuchat, 2005) The cultures were prepared and incubated as were the cultures of *E. coli*. Portions of each culture were diluted with peptone water. The diluted cultures were mixed to obtain suspensions with approximately equal numbers of each strain that together gave the numbers required for inoculation.

2.2. Determination of the acid resistance of bacteria in inocula

Suspensions of *E. coli* and the *E. coli* O157:H7 cocktail, at numbers of about 6.5 log cfu/ml were prepared. A 6 ml volume of each suspension was added to an equal volume of each of 16%, 8%, 4% and 2% L + lactic acid (Sigma–Aldrich, Mississauga, Ontario, Canada). Each mixture was incubated for 30 min before two 1 ml portions were withdrawn. Each portion was used for the preparation of serial ten-fold dilutions in buffered peptone water (Difco), to 10^{-4}. Subsequently, at 30 min intervals up to 120 min, two 1 ml portions were similarly withdrawn and diluted. All suspensions, diluents and mixtures were maintained at 10 °C in a shaking water bath.

The whole 9 or 10 ml of each dilution in each series prepared at each time was filtered through a hydrophobic grid membrane filter (HGMF; Oxoid, Mississauga, Ontario, Canada). For mixtures containing *E. coli*, the filters used with one series of dilutions prepared at each time were each placed on a plate of lactose monensin glucuronate agar (LMG; Acumedia, Lansing, MI, USA). The filters used with the other series of dilutions were each placed on a plate of LMG supplemented with bile salts (Sigma–Alderich) at 1.5 g/L (LMGB). For mixtures containing *E. coli* O157:H7, the filters used with one series of dilutions prepared at each time were each placed on a plate of sorbitol McConkey agar (SMAC; Difco) supplemented with potassium tellurite (Sigma–Aldrich) and cefixime (Sigma–Aldrich) at 2.5 mg/l and 0.05 mg/l, respectively (CT-SMAC). Filters used with each of the other series were each placed on a plate of plate count agar (PCA; Difco) and incubated at 35 °C for 3 h. The filter was then transferred to a plate of CT-SMAC. LMG allows resuscitation of injured *E. coli* while incorporation of bile salts in the medium prevents resuscitation of injured cells (Entis & Boleszczuk, 1990; Merritt & Donaldson, 2009). CT-SMAC does not allow resuscitation of injured *E. coli* O157:H7. Incubation on a non-selective medium, such as PCA, before exposure to the selective agar allows resuscitation of injured *E. coli* O157:H7 (Blackburn & McCarthy, 2000).

All plates of LMG, LMGB and CT-SMAC were incubated at 35 °C for 24 h. Then, square containing colourless colonies on filters on plates of CT-SMAC were counted. Filters on plates of LMG or LMGB were transferred to plates of buffered 4-methylumbelliferyl-β-D-glucuronide agar (BMA; Acumedia), which were incubated at 35 °C for 3 h. The filters were illuminated with long wave length UV light, and squares containing blue-white, fluorescent colonies were counted. Squares containing colonies were preferably counted on filters bearing between 20 and 200 of the targeted colonies. A most probable numbers (MPN) of *E. coli* O157:H7 or *E. coli* was calculated from each count of squares using the formula $\mathrm{MPN} = N \times \log_n (N/N{-}X)$, where X is the total number of squares on a filter and N is the number of squares containing the targeted colonies.

2.3. Equipment used for treating beef surfaces

The equipment used for treating beef surfaces has been described (Youssef et al., 2012). Briefly, the equipment consisted of a wire mesh conveyor belt which carried meat slices through a fan spray of fluid delivered across the width of the belt. The belt was driven by a variable speed motor. The line to the spray nozzle was connected to a line in which the fluid to be sprayed was circulated from a reservoir. A valve in the fluid circulation line allowed the fluid pressure at the spray nozzle to be adjusted, to deliver fluid from the nozzle at a required rate.

The meat to be treated was packed into plastic trays with internal dimension of 23 × 15 × 1.5 cm, in a 1 cm deep layer in each tray. The height of the spray head above meat surfaces was fixed. The volume of fluid sprayed onto surfaces was controlled at 0.5 ml/cm^2 by adjustment of the valve in the fluid circulation line and the speed of the conveyor belt.