CANADIAN MEAT SCIENCE ASSOCIATION

Growth of *Escherichia Coli* at Chiller Temperatures

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Introduction

The responses of mesophilic microorganisms to chiller temperatures depend on the conditions under which the organisms are exposed to such temperatures. While the effect of cold temperatures on the viability of microorganisms that are subjected to sharp drops in temperature (cold shock) has been well documented, there is a lack of information on the effect of chiller temperatures on cells that are cooled relatively slowly. Temperatures of chilled foods may fluctuate above and below the minimum growth temperature of *Escherichia coli* and other related mesophilic pathogens. *E. coli* can grow exponentially on raw meat when temperatures remain or rise above 7°C during carcass cooling, fabrication of cuts, and display. The current assumption is that *E. coli* does not grow below 7°C. Models for predicting the growth of bacteria in foods have been developed by monitoring changes in the absorbances of or the numbers of colony forming units (cfu) recoverable from liquid cultures. When models that relate the durations of lag phases and the growth rates of bacteria to the physical and chemical conditions of growth media are constructed from such data, it is necessarily assumed that the population of cells in each culture is homogeneous. While that may often be the case, the assumption is not always correct (McKellar, 1997).

Incubation at chiller temperatures can result in abnormal cell growth

In a recent study of the behaviour of cold adapted, log phase E. coli cultures during incubation at temperatures below the minimum for sustained growth, and subsequent return to a growth permitting temperature, it was found that the mean lengths of cells remained constant when cultures were incubated at 2°C (Fig. 1). The cultures proceeded to sustained exponential growth during subsequent incubation at 12°C (Jones et al., 2002). Such findings are agreeable with the general expectation of homogeneity within a culture. However, when cultures were incubated at 6°C, substantial fractions of the cells elongated. When those cultures were subsequently incubated at 12°C, the elongated cells further increased in length before they divided into cells of normal size (Fig. 2). During the time that elongated cells were dividing, the numbers of cfu recovered from cultures increased at rates greater than the exponential growth rate of homogeneous cultures at 12°C. Cultures that were incubated at 7°C behaved similar to those incubated at 6°C with absorbances and cell lengths increasing while the numbers of colonies recovered were at first

unchanged, and then declined (Figs. 3 and 4). Evidently, 7°C is below the minimum temperature for sustained growth of the strain of *E. coli* used in these studies (Jones et al., in press); however, growth was sustained at 8°C. Despite that, a large fraction of the cells elongated and the relationship between absorbance and numbers of viable cells altered as growth proceeded at 80°C. As a result, the growth rate estimated from absorbance values would be more than double the rate that would be estimated from the numbers of cfu. At incubation temperatures of 9°C and above, elongation of cells did not generally occur during the first day of incubation. Consequently, with data collected during that time, growth rates estimated from absorbance values or numbers of cfu would be similar, as is generally expected (McMeekin et al., 1993). However, when incubation at temperatures between 9 and 12°C inclusive was extended beyond 1 day, the cultures became increasingly heterogeneous in cell size. The population of cells was homogeneous when cultures were incubated at 15°C.

E. coli cells, when transferred from 37°C to 10°C, are reported to adapt to cold temperatures within 4 h (Jones et al., 1987). However, the cold adaptation process is apparently more complex than has been understood, as a subpopulation of the bacterial cells showed distress by elongating after a prolonged period of time at temperatures as high at 12°C.

Implications of the presence of elongated cells

Obviously, models based on either absorbance or plate count data, with the assumption of culture homogeneity, could not accurately describe the behaviour of *E. coli* at temperatures that fluctuate about the minimum for growth (Gill et al., 2001). Increases in cell number or biomass contribute to increases in absorbance values. The linear relationship between cell numbers and absorbance is invalid when cells experience conditions of unsustained growth or death. However, bacterial growth models based on enumeration of cells without taking account of increases in biomass may result in underestimation of risks arising from bacterial proliferation at temperatures below the minimum for sustained growth, because elongated cells may rapidly divide upon return to favourable growth conditions. Difficulties are also encountered in the modeling of lag phase durations and bacterial growth when factors such as temperature, pH and a_w approach the limits for growth. The lack of fit of bacterial growth data to existing models could be partially due to the formation of elongated cells under conditions of low a_w and starvation as well as a low temperatures (Wainright et al., 1999; Mattick et al., 2000).

Growth by elongation when environmental conditions approach the limits for growth may lead to a misunderstanding of the health risks associated with the number of some pathogens recovered from foods, because each elongated cell would be detected as a single colony with consequent underestimation of the potential numbers of bacteria to which consumers would be exposed when elongated cells rapidly divide at room or body temperatures. For example, when *Salmonella* was incubated at 8°C, filaments up to 150 µm long were formed. When the culture was warmed

to 37˚C, a 200-fold increase in cell counts was observed without an accompanying increase in biomass (Mattick et al., 2003).

Although an increase in bacterial numbers from elongated cells may be of little significance with organisms that have high infective doses, even small increases in the numbers of pathogens with very low infectious doses, such as *E. coli* O157:H7, may substantially increase risks to consumers' health. As the behaviours of pathogens at temperatures near their minimum for growth can be complex, current assessments of microbiological risks associated with chilled foods might well be erroneous, irrespective of whether they are based on the predictions of "fail safe" models (Little and Knøchel, 1994) or direct determination of increases in the numbers of colony forming units in foods.

Future directions

A study evaluating the behaviour of *E. coli* under fluctuating temperatures is currently under way. Preliminary results have indicated that filamentous cells are able to divide when the temperature rises briefly from blow to above the minimum growth temperature. Further studies will be needed to determine if the findings for broth cultures can be extended to describe the behaviour of *E. coli* in chilled foods. As filamentation occurs in response to stress, it is necessary to determine whether filamentous cells have increased resistance to other stresses or if they respond in the same manner as cells of normal size growing at chiller temperatures. The physiological response to stress can be detected by changes in the protein expression pattern. Cultures entering the lag phase at 2˚C and at 6˚C are currently being evaluated for changes in their protein expression patterns. An understanding of the physiological, biochemical, and molecular mechanisms involved in responding to cold temperatures is essential for predicting microbial growth and for identification of effective methods for controlling the growth of pathogenic bacteria in chilled foods.

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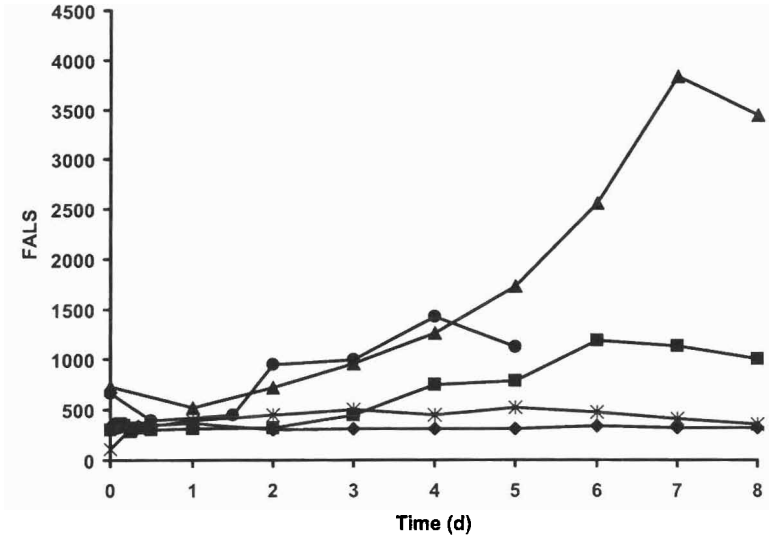

Fig. 1. Mean Foreward Angle Light Scatter (FALS) flow cytometry measurements of the longest 10% of cells in cultures of log phase *E. coli* incubated at 2°C(◆), 6°C(■), 7°C(▲), 12°C(●) or 15°C(✳).

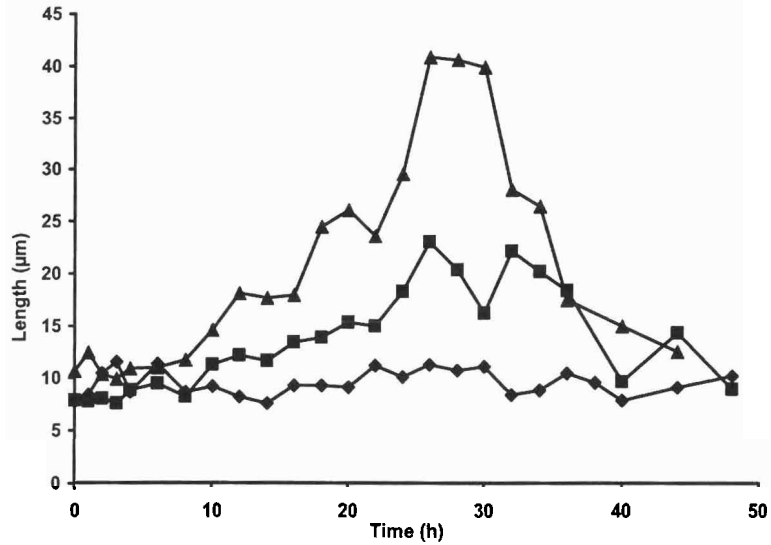

Fig. 2. Mean lengths of the longest 10% of cells in cold adapted, log phase *E. coli* cultures incubated at 12°C for 48 h after incubation at 2°C for 4 days (◆) or after incubation at 6°C for 4 days(■) or 8 days(▲).

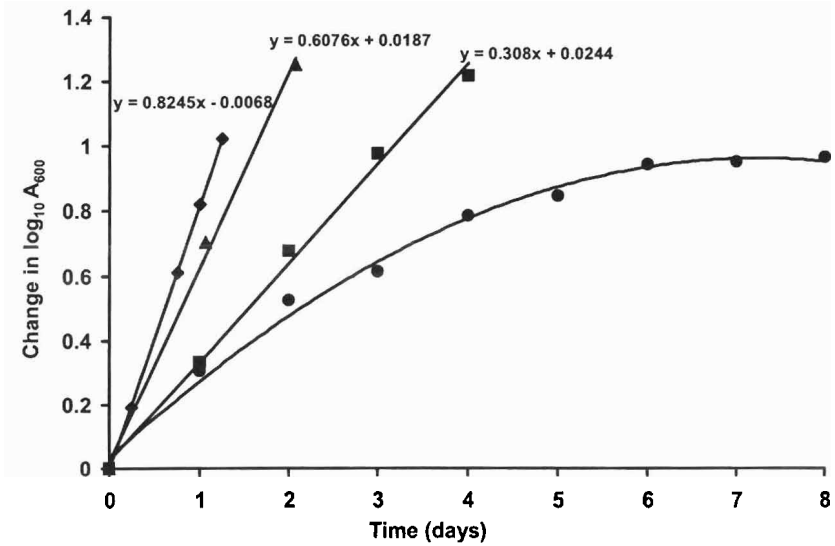

Fig. 3. Changes in absorbance values of cold adapted, log phase *E. coli* cultures incubated at 7°C(●), 8°C(■), 9°C(▲) or 10°C(◆).

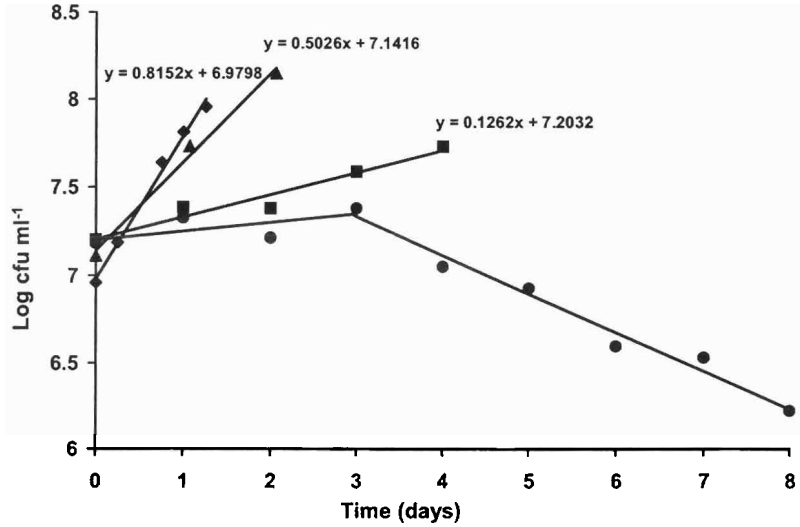

Fig. 4. Numbers of *E. coli* (log cfu ml^{-1}) recovered on PCA from cultures of cold adapted, log phase cells incubated at 7°C(●), 8°C(■), 9°C(▲) or 10°C(◆).