Lag Phase of *E.coli*, *Salmonella* spp. and *S. aureus* cells on meat at different temperatures

by Pattis I, Horn B and Rivas L (2017)

LAG PHASE

Definitions

A lag phase can be defined as the amount of time required for a bacterial cell to adjust to a new environment prior to replication (growth). In the PHI context, for example, this would be the time required to adjust to the conditions on the carcass surface due to movement of faecal matter onto the dressed carcass surface (Mills 2012) or change to anaerobic conditions. However, the lag phase is the most unpredictable part of microbial growth as its duration strongly depends on the previous growth conditions of the microorganism.

This is because the previous environment of the bacteria will determine the cellular changes that need to be made before the organism can grow in a new environment. For example, the lag phase duration of bacteria grown at 37°C in culture media and then transferred to a meat surface at 20°C will be different than the lag phase duration of bacteria grown at 25°C and then transferred to a meat surface at 20°C. Generally, the lag phase is shorter if the inoculum is more metabolically active, e.g. in the exponential growth phase than in the stationary phase where metabolic activity is low (Montville and Matthews, 2001).

As a result, it can be challenging if not impossible task to compare lag time results from different experimental settings. To partly overcome this limitation, lag times can be 'normalised' to the growth rate[1], or equivalently the generation time, that can be achieved by the cells in that specific environment (Robinson 1998, Ross 1999, Mellefont 2003):

Lag time / generation time = "relative lag time" or "generation time equivalent"

That is, the ratio of the lag time divided by the generation time is a measure of how much 'work' a bacterial cell has to do before it can initiate growth. This ratio is called the 'relative lag time' or 'generation time equivalent'.

In a predictive growth model, the effect of lag time on the predicted growth of microorganisms can be incorporated and the relative lag time distribution can be subtracted from the predicted growth rate distribution. The effect is a reduction of predicted generations.

DATA SOURCES

This section compares data on the lag phase for *E. coli*, *Salmonella* spp. and *S. aureus*. There was very limited fresh meat growth data in the relevant temperature range identified from an initial search of the literature. Only one study on intact meat, covering a wide range of growth temperatures and an adequate experimental setup was identified (Dickson 1992). However, this study only considered a single strain of *Salmonella* Typhimurium.

[1] The **growth rate** is the change in bacterial numbers over some period of time, typically expressed as $\log_{10}$ per hour. The **generation time** is the time (usually stated in hours) that it takes for one cell to divide and become two cells. To convert generation time to growth rate, divide 0.301 (the $\log_{10}$ value of 2) by the generation time.

Table 1 below provides an overview of the experimental details of three different studies which provided the majority of experimental data for this section (Smith 1985, Dickson 1992, Ingham 2007).

Table 1: Experimental details of studies presented in this fact sheet.

Study	Meat	Meat sourced from	pH	Inoculum	Inoculation Temp.	Conditions
Smith 1985	Blended mutton tissue[a]	abattoir	5.7–6.3	*E. coli, Salmonella* grown to stationary phase at 37°C or for coliforms, meat rubbed over anal area of animal meat sample came from	10, 15, 20, 25, 30, 35, 40°C in water bath	Vacuum sealed thin films of inoculated meat in PVC pouches, oxygen permeable (aerobic)
Dickson 1992	Intact beef tissue (lean & fatty) Sterile	abattoir	5.6-6.2	Tissue immersed in *Salmonella* suspension (grown at 37°C until late log. growth phase)	15, 20, 25, 30, 35, 40°C and analysed at 2-hr intervals.	hung in sterile container (aerobic)
Ingham 2007	Ground pork and beef	retail store	5.4–5.7	Five strains each of: *E. coli* *Salmonella* *S. aureus* (grown at 35°C to stationary phase)	10 – 43°C (at 2.8°C intervals)	25 g meat in sample bags (7.5x18.5 cm)

[a] Blending the meat tissue ruptures the muscle cells, releasing nutrients and moisture available for the bacterial metabolism. Thus, blending might reduce the length of time bacteria require to adjust to the environment, hence resulting in a shorter lag time (Dickson 1992).

GENERATION TIME EQUIVALENTS

Ross (1999) provides a comprehensive report on predictive microbiology for the meat industry and conducted a literature search on published growth rates and lag times of selected microorganisms in broth, foods and meat; all results were compiled and presented as histograms. The author points out that although 'lag times may take almost any value' there is a 'common distribution of relative lag times with a sharp peak in the range of 4-6 generation time equivalents. The distribution curve of lag times of *E. coli* growing on foods shows a sharp peak at three generation equivalents whereas *E. coli* growing in broth was found to show a distribution curve of lag time peaking at around five generation time equivalents. Analysis of growth data of *Salmonella* in meats resulted in a distribution curve of lag times with a peak around five generation time equivalents. However, we were not able to access the original data sets that were used for the calculations which limits further interpretation as product type, time, temperature, pH and cell history is not known.

The Australian RI allows for a lag phase of five generations in bacterial growth for meat that starts as 'hot or warm'. The choice of five generations was based on a study by Smith (1985) and the survey by Ross (1999) which utilise published data and experimental results:

Smith (1985) describes a lag time duration of 4.6 times of the expected generation time at 40°C. However, at lower temperatures the duration of the lag phase decreased significantly (3.2 times at 35°C and 2.9 times at 30°C).

Figure 1 below plots experimental lag time data points derived from the three different studies summarised in Table 1 (Smith 1985, Dickson 1992, Ingham 2007). The lag phase duration is presented as generation time equivalents. For ground meat studies, *E. coli* data is represented by triangles, *Salmonella* spp. data by circles and *S. aureus* data by diamonds. The Salmonella data for intact meat is represented by stars.

Due to the limited number of studies and data points presented, it is necessary to interpret trends with caution. In the temperature range 30-40°C, the temperature range of relevance to after slaughter and dressing, the shortest generation time equivalent of lag is 2 generations and the longest is close to 10 generations. For the intact tissue studies of Dickson (1992) the lag time was in the range of 5 to 7 generation equivalents for the temperature range 30-40°C. At lower temperatures, the generation equivalents of lag remained constant at 5 generations for lean beef, but increased with decreasing temperature for fatty beef.

The coliform data (Smith 1985) which used experiments that inoculated the meat by smearing the meat tissue on the anal area of the carcass, is the closest experimental set up to processing contamination. The generation time equivalent of lag for the coliform data is all in the range of 2 to 3.5 generations.

The validity of incorporating a lag phase following dressing for growth predictions, may be dependent on the state of the cows just before slaughter. Theoretically, if the *E. coli* on the animals come from dried faeces on hides and are in a desiccated state, there is more likely to be a lag in growth when they end up on the tissue surface, than if *E. coli* are coming from wet faeces. However this would need to be verified experimentally.

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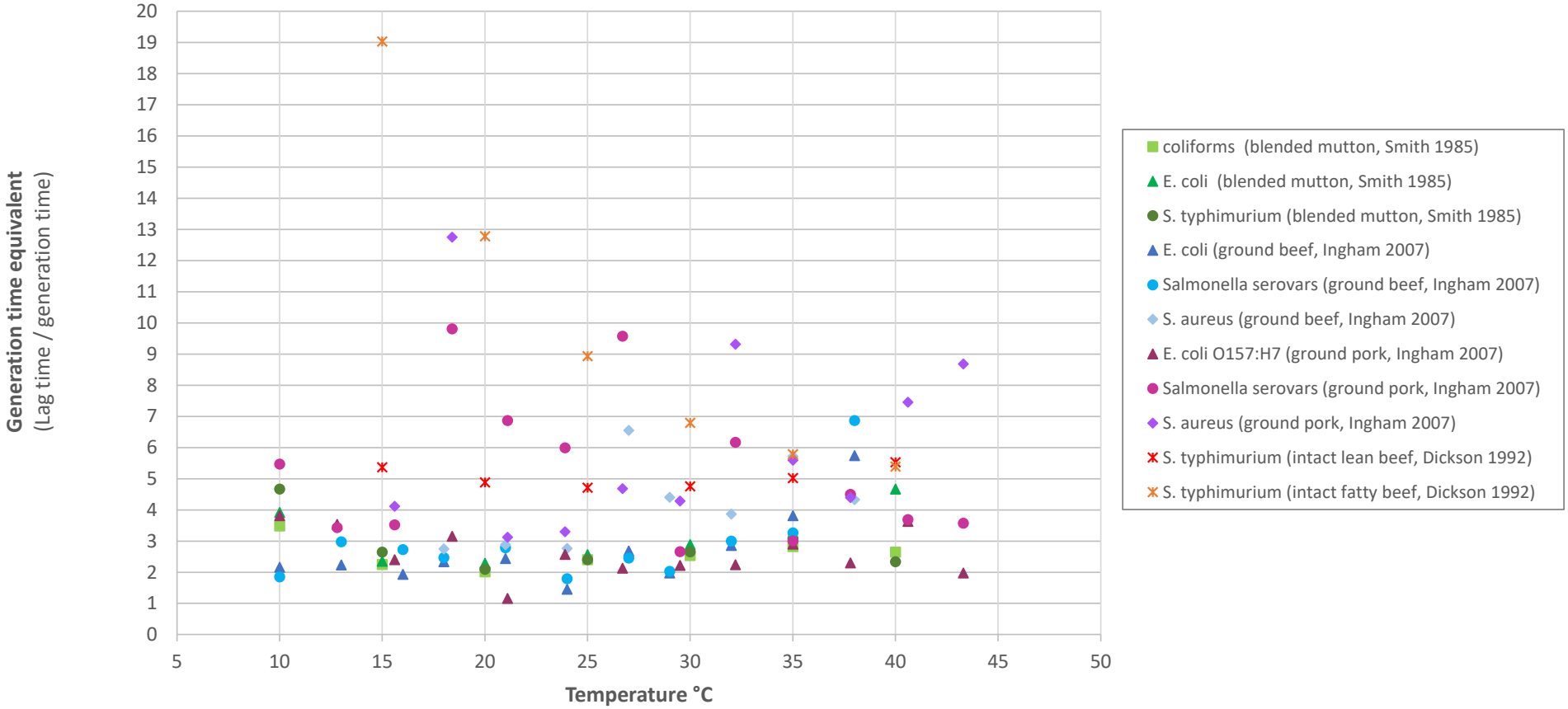

Figure 1: Generation time equivalents of lag phase duration of experiments with different meat types, bacteria and pathogens.