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Haumaru Kai Aotearoa

Growth of microorganisms in raw milk: Evaluating the effect of chiller failure

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Scientific Interpretative Summary

This SIS is prepared by New Zealand Food Safety (NZFS) risk assessors to provide context to the following report for NZFS risk managers and external readers.

Growth of microorganisms in raw milk: Evaluating the effect of chiller failure

Adequate milk cooling is essential for ensuring quality milk is supplied. Therefore, time/temperature requirements standard for the cooling of milk are set in the Operational Code: NZCP1: Design and Operation of Farm Dairies. Milk that has not been cooled in accordance with the Code requirements "must be withheld from supply, unless the milk has been assessed and confirmed as fit for intended purpose by the RMP Operator / dairy company through measures such as: a) sensory evaluation; b) microbiological testing; c) titratable acidity; or d) a validated risk assessment model.

NZFS commissioned a project to inform risk models by providing estimates on expected bacterial growth for different scenarios of chiller failure. The intent of this was to enable mechanisms to be put in place that facilitate faster management decisions around acceptance/rejection criteria for raw milk, with an overall objective to limit the quantity of milk discarded unnecessarily.

The report provides a comprehensive review of available data and growth models for a range of pathogenic and spoilage bacteria that might be present in raw milk. For each microorganism considered in this project a substantial variability in growth rates between studies and strains has been noted.

Raw milk itself contributes to the variability of growth rates as it is not a microbiologically consistent substrate, with potentially different microflora in different samples which may inhibit or promote the growth of pathogens or spoilage organisms. Therefore, a conservative approach, when growth models are calibrated using data on fast growing strains from heat treated milk studies, has been defined. This approach uses maximum growth rate, with or without an added safety margin, to consider different scenarios of chiller failure defined by the time of chiller failure and the maximum temperature the milk reaches during the failure.

The outcome of this work is guidance on the potential of generic micro-organism growth during chiller failure. Use of this guidance enables a robust estimate around the probability milk is not fit for purpose and, consequently, informed decision making on acceptance/rejection of raw milk.

Growth of microorganisms in raw milk: Evaluating the effect of chiller failure

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Introduction

Milk that is collected on-farm is cooled in milk vats at the farm until collection by the processor's milk tankers. Periodically, farm vat refrigeration or power failures can occur, resulting in the loss of active cooling of the milk, leading to increases in the milk temperature, until the cooling recommences.

This report has collated and evaluated data and growth models for *Lactic acid bacteria, Pseudomonas* spp., *Bacillus cereus, Campylobacter* spp*., Clostridium* spp., *Listeria monocytogenes, Escherichia coli, Salmonella* spp*.* and *Staphylococcus aureus* in raw and heat-treated milk for the temperature range 4 to 20°C.

This information has been collected to help inform the risk management question: How far from the currently prescribed time-temperature parameters can milk cooling deviate before there is an increased risk from microbiological hazards and spoilage organisms that would preclude the milk from entering the food chain?

Data

This review of microbial growth in milk has found gaps in currently available knowledge on growth rates in the temperature range of 4° to 20°C for all the microorganisms considered in this report.

There are limited data available on growth rates of microorganisms in raw milk. The studies that have been conducted to determine the parameters of the primary growth models (lag time, maximum growth rate and maximum cell density) have been conducted in heat-treated milk with the exception of one *L. monocytogenes* study.

Most of the studies using raw milk are focused on longer storage times, and typically the first sampling is 16 to 24 hours after incubation. Where comparison of the growth rate of microorganisms in raw and heat treated milk is possible the growth rate in raw milk is at the lower range of growth rates in heat treated milk, with the exception of *S. aureus* where it was similar.

For each microorganism, there are limited data on maximum growth rates in different strains in the temperature range of 4 to 20°C. *S. aureus* and *L. monocytogenes* have the most data available. However, even for these microorganism s more data, especially relating to growth in raw milk, may be required to provide suitable evidence for risk assessment.

Growth Models

Raw milk is not a microbiologically consistent substrate, with potentially different microflora in different samples which may inhibit or promote the growth of pathogens or spoilage organisms. All but two of the described growth models are specific to the laboratory experiments used to calibrate them. The limited data available makes it impossible to properly evaluate any of the proposed growth models for risk assessment, given the expected variability between study, strain growth rates and the natural microflora of the raw milk.

The models that were designed to recognise study, strain or raw milk variability used two different approaches. Neumeyer et al. (1997) used growth studies in broth to calibrate the model, but used a strain which they had identified as a fast growing for the range of temperatures considered. The use of this strain provided an estimate of the greatest likely

amount of growth. The growth rates predicted by this model are greater than all the observed growth rates selected during the project.

Augustin et al. (2005) recognised the variability in growth rates observed between studies and strains, and fitted bounds on the expected growth rates, based on the data from 14 different liquid dairy product studies. Based on the currently available data, both these models have the potential to be useful to predict maximum grow rates, however growth data for a much wider range of different microorganism strains would be needed to validate the models further.

Scenarios

There are insufficient data to produce or validate models for estimating the maximum growth of specific microorganism s at given temperatures in raw milk. To provide some initial guidance on the potential of generic microorganism growth during chiller failure, a scenario approach based on the data that have been able to be collected is presented.

This approach uses the whole dataset to provide a microorganism generic boundaries for the maximum growth rate which is temperature dependent. The maximum growth rate can be applied, with or without an added safety margin, to consider different scenarios of chiller failure defined by the time of chiller failure and the maximum temperature the milk reaches during the failure.

1. INTRODUCTION

Milk that is collected on-farm is cooled in milk vats at the farm until collection by the processor's milk tankers. Periodically, farm vat refrigeration or power failures can occur, resulting in the loss of active cooling of the milk, leading to increases in the milk temperature, until the cooling recommences.

If the temperature abuse occurs for a short period of time, the milk destined for pasteurisation may remain fit for purpose despite having breached milk cooling requirements. Yet, under the current requirements this milk may be rejected for collection.

1.1 REGULATIONS

Dairy farms in New Zealand must follow Operational Code NZCP1: Design and Operation of Farm Dairies with respect to milk collection and storage processes. Section 5.15 states that,

"(2) Raw milk must:

a) be cooled to 10°C or below within four hours of the commencement of milking;

b) be cooled to 6°C or below within the sooner of:
   i) six hours from commencement of milking
   ii) two hours from the completion of milking; and

c) be held at or below 6°C without freezing until collection or the next milking; and

d) must not exceed 10°C during subsequent milkings

(3) In situations where there is continuous or extended milking, such as automated milking systems, the milk must enter the bulk milk tank at 6°C or below. "Continuous or extended milking" is defined as milking for six hours or longer from the time the milk first enters any bulk milk tank."

Currently the requirements given in 5.15(2) must be implemented for new dairies or dairies which are undergoing significant changes to the secondary milk cooling system. From 1st June 2018 all dairies must meet the requirements in 5.15(2).

Section 5.16(2) of NZCP1 states:

"Milk that has not been cooled in accordance with clause 5.15 must be withheld from supply, unless the milk has been assessed and confirmed as fit for intended purpose by the RMP Operator / dairy company through measures such as:

a) sensory evaluation
b) microbiological testing
c) titratable acidity; or
d) a validated risk assessment model."

1.2 PROJECT AIMS

The purpose of this report is to collate and evaluate data and growth models for pathogenic and spoilage bacteria in milk for the temperature range 4 to 20°C. This information will help inform the risk management question: How far from the currently prescribed time-temperature parameters can milk cooling deviate before there is an increased risk from microbiological hazards and spoilage organisms that would preclude the milk entering the food chain?

2. PROJECT METHODOLOGY

2.1 REVIEW

A review of data and models was conducted by:

- A systematic literature review on growth data and predictive models in the temperature range from 4 to 20°C was conducted. Details on the search strategy and inclusion/exclusion criteria are provided in Appendix A.
- A review of data in Combase[1] for relevant data that have not been published in the open literature.
- A review of past ESR experimental work and risk assessments.

The following microorganisms, representing the most relevant pathogens and spoilage organisms in raw milk, were included:

- *Lactic acid bacteria*
- *Pseudomonas* spp.
- *Bacillus cereus* (*B. cereus*)
- *Campylobacter* spp.
- *Clostridium* spp.
- *Listeria monocytogenes* (*L. monocytogenes*)
- *Escherichia coli* (*E. coli*)
- *Salmonella* spp.
- *Staphylococcus aureus* (*S. aureus*)

Due to the limited data available for growth of microorganisms in raw milk, data corresponding to a range of heat treated milk, such as pasteurised, ultra-high temperature processed (UHT) or sterilised milk were also collected. The milk types are identified in the presentation of data.

This report provides available information on each of the above microorganisms:

- Potential growth of microorganism in milk at constant temperatures.
- Growth of microorganisms under temperature changes similar to those experienced during a refrigeration failure.
- Any growth models that have been developed using milk or tested against milk.

The rate of growth of microorganisms in milk subjected to constant temperatures has been collected in two ways: maximum growth rates and observed growth after a given time period.

Maximum growth rates have been extracted from the papers where there is sufficient evidence present to define the shape of the growth curve and therefore identify the logarithmic growth phase.

In other papers, where there is evidence of growth or no growth, but insufficient data to calculate a maximum growth rate, this information is summarised in the text, and tables are provided in the Appendix B.

[1] www.combase.cc (Accessed 14th July 2017)

2.2 GROWTH NOTATION

In the published literature there is inconsistent use of symbols to represent growth rates during exponential growth (Appendix C) , with μ and k being used for growth rate constants with different definitions. For the purposes of this report the following will be used:

k : Specific growth rate [h^{-1}], Generation time [h] = (ln 2) / k

μ_{max} : Exponential growth rate [Log_{10} CFU/mL hour] Generation time [h] = (Log_{10} 2) / μ_{max}

3. LACTIC ACID BACTERIA

Lactic acid bacteria (LAB) are part of the autochthonous microbiota of milk. LAB represent an order of bacteria that share common metabolic and physiological characteristics. Naturally found in milk products and decomposing plants, they produce lactic acid as the main metabolic end product. Common LAB include the genera *Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus* (Franciosi *et al.*, 2009).

Optimum growth temperatures of LAB range from 30 – 45°C (Gilmour and Rowe, 1990).

3.1 GROWTH

3.1.1 Single Temperature Growth

Examination of the literature did not result in many papers with data within the project scope. Maximum growth rates for temperatures between 9 and 21°C were calculated by (Acai *et al.*, 2016) for the Fresco culture DVS 1010 (*Lactococcus lactis* subsp.*lactis* and *cremoris, Streptococcus thermophilus*) innoculated into UHT milk and by (Valbuena *et al.*, 2008) for *Lactococcus lactis* subsp.*lactis* in reconstituted skimmed milk. The estimated maximum growth rates are given in Figure 1.

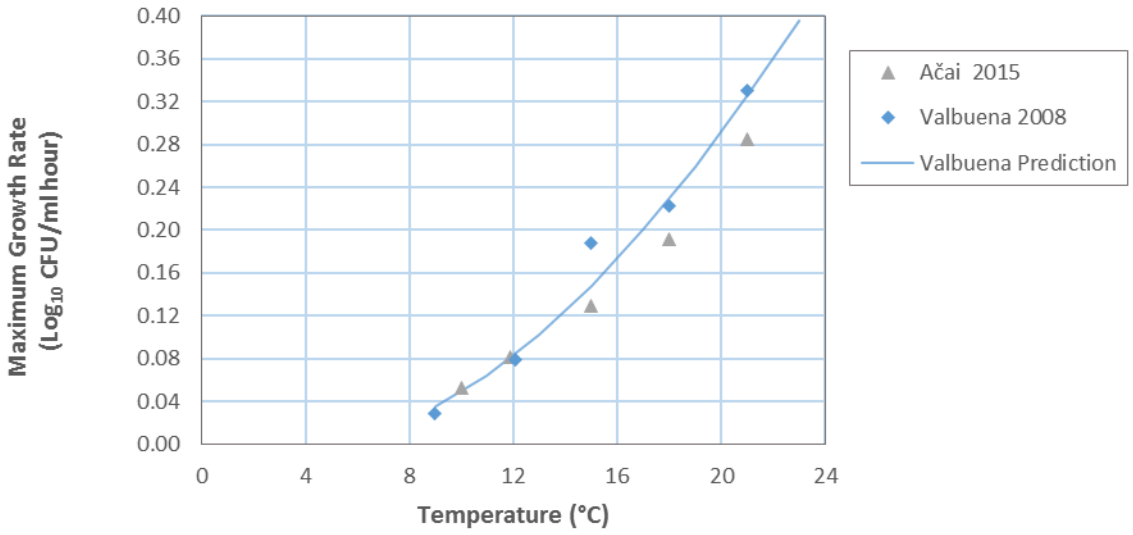


Figure 1 Lactic acid bacteria maximum growth rates in heat treated milk from logarithmic growth phases at different constant temperatures from 9 to 21°C compared to the model of Valbuena et al (2008).

Two papers reported growth studies of naturally occurring lactic acid bacteria in raw milk. At temperatures of 4-6°C no growth was observed after 24 hours (Malacarne *et al.*, 2013; Zhang *et al.*, 2008).

Growth of lactic acid bacteria was observed in whole cow's milk incubated at 8-10°C and 13-15°C, with approximately 0.7 and 2 Log_{10} CFU/ml of growth observed after 24 hours respectively. In raw donkey's milk incubated at 20°C no growth was observed after 8 hours, but 1.5 Log_{10} CFU/ml growth was observed 16 hours after the start of incubation (average growth rate 0.094 Log_{10} CFU/ml hour).

3.2 GROWTH MODELS

Valbuena et al (2008) fitted a Gompertz model to their experimental data, incubating *Lactococcus lactis* subsp.*lactis* in reconstituted skimmed milk at different fixed temperatures. They then fitted the estimated Gompertz parameter values (B,D,M and N_0) as functions of temperature in the range 9 to 39°C.

The resulting estimate for the maximum log growth rate is given by the equation

$$\mu_{max} = BD/e^1$$

where

$$\sqrt{B} = [0.021\ (T - 3.648)]\left[1 - e^{(0.399\{T-42.704\})}\right]$$

$$D = \frac{671.206}{T + 79.975}$$

This model fits the experimental data well (Figure 1), but is for a single strain of *Lactococcus* in sterilised reconstituted milk. D is a function of temperature, with the maximum population density decreasing with increasing temperature. The D parameter estimates of the Gompertz equation plotted against temperature were variable around a decreasing trend. The lower and upper bands of the D estimates, had a range of approximately 1 Log_{10}.

3.3 CONCLUSIONS

Minimal data on the growth of LAB in raw milk incubated in the temperature range 4 to 20°C are available in the literature. The two raw milk growth experiments described above have lower growth rates than the two experiments using inoculated sterile milk, however more data would be required to confirm whether this is true in general.

For the data that are available, maximum growth rates of 0.05, 0.19 and 0.33 Log_{10} CFU/ml hour at 10, 15 and 21°C have been observed.

A model for growth based on the Gompertz function has been fitted for a single strain of *Lactococcus lactis.* The model would need to be tested against more strains and raw milk, to evaluate its usefulness.

4. *PSEUDOMONAS* SPP.

The genus *Pseudomonas* represents the majority of psychrotropic bacteria in milk. One strain, *P. fluorescens,* is considered the main spoilage agent of stored milk (de Oliveira *et al.*, 2015).

During late exponential to early stationary phase of cell growth, protease and lipase activity can result in the release of enzymes. Sufficient enzymes to cause spoilage are usually present if the cell population reaches 10^6 – 10^7 CFU/ml (Frank, 2007).

Although the bacteria themselves are killed by pasteurisation, their enzymes are often heat-resistant and can retain activity after heat treatment. This can lead to spoilage of milk after pasteurisation or render milk unsuitable for the production of certain dairy products.

The optimum temperature range for growth of *P. fluorescens* or *P. putida* is 25 to 30°C, while the optimum growth temperature for *P. aeruginosa* is higher at 37°C (Gilmour and Rowe, 1990).

4.1 GROWTH

4.1.1 Single temperature growth

The rate of growth of *Pseudomonas* spp. in milk subjected to a constant temperature has been collected in two ways: maximum growth rates and observed growth after a given time period.

For studies which have sufficient sample time points to allow the maximum growth rate to be calculated, the data are presented in Figure 2. There is variability between studies, which is likely to be due to the different strains used in the different studies. At the plotted growth rates, an 1 Log_{10} CFU/ml increase in cells would take approximately 10 to 16 hours at 10°C, and 5 to 6 hours at 20°C.

The three studies (Greene and Jezeski, 1954; Lin *et al.*, 2016; Shelley *et al.*, 1986) all used heat treated milk as the substrate.

Another study, compared the growth rate of 2 *P. fragi* and 7 *P. fluorescens* strains at 4°C in raw goat milk, or the same raw milk which had been UHT treated (Cox and MacRae, 1988). The growth rates of the one *P. fragi* strain did not differ between the raw and UHT treated milk experiment, for the other strain, the growth rate was 0.02 Log_{10} CFU/ml hour greater in UHT treated milk compared to raw milk. The *P. fluorescens* strains growth rates in UHT treated milk were between 0.02 and 0.05 Log_{10} CFU/ml hour greater than in raw milk. This suggests at lower temperatures (4°C), more *Pseudomonas* growth would be expected in heat treated milk compared to raw milk.

One other study used 3 goat milk strains of *P. fluorescens* inoculated into raw goat milk (Zapico *et al.*, 1995). The log increase in cells after 24 hours, was approximately 0.5 and 1 Log_{10} CFU/ml at 4 and 8°C respectively. This is equivalent to an average growth rate of 0.02 and 0.04 Log_{10} CFU/ml hour, which corresponds to the lower bound of the data in Figure 2.

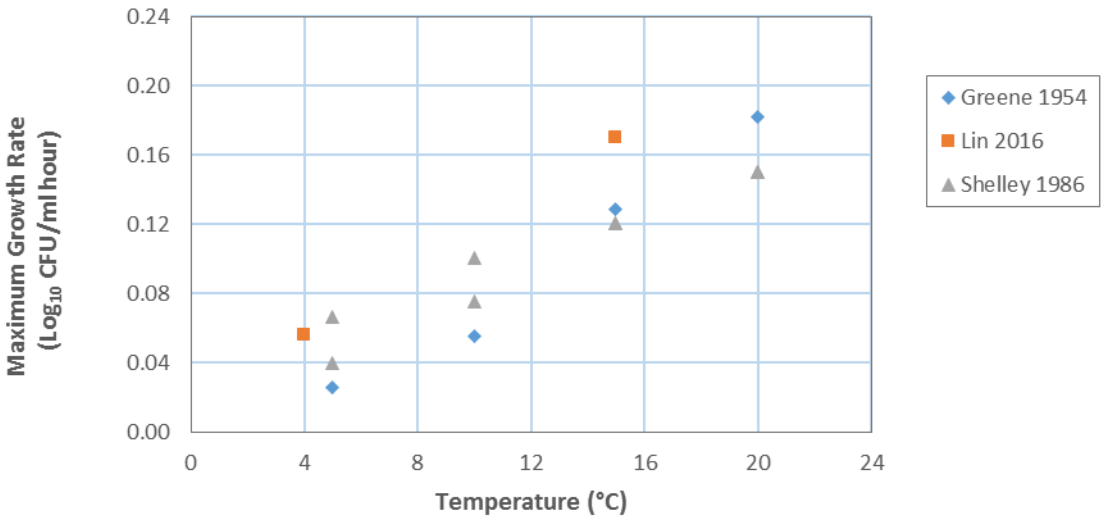


Figure 2: *Pseudomonas* spp. maximum growth rates in heat treated milk from logarithmic growth phases at different constant temperatures from 4 to 20°C.

4.1.2 Naturally contaminated milk

Two constant temperature studies used raw milk with no additional inoculation of *Pseudomonas* spp. (De Jonghe *et al.*, 2011; Giacometti *et al.*, 2016). At 4 to 6°C minimal growth of *Pseudomonas spp.* (< 0.5 Log_{10} CFU/ml) was observed after 24 hours in cow, donkey or goat milk. At an incubation temperature of 12°C, *Pseudomonas* spp. grew by 1.5 Log_{10} CFU/ml after 24 hours in donkey milk.

These growth patterns correspond to the lower bounds of the maximum growth rates given in Figure 2.

4.1.3 Multiple temperature growth

Lin et al. (2016) conducted experiments with *P. fluorescens* in UHT milk, for a two temperature profile; 24 or 48 hours at 4°C, followed by 6 hours at 15°C, then returned to 4°C.

A 0.5 Log_{10} CFU/ml growth was observed after the initial 24 hours at 4°C. The 6 hours at 15°C increased the cell population be a further ~1 Log_{10} CFU/ml. No lag in growth rate was observed after the sudden temperature change, and the growth achieved during the 15°C step, was at a similar rate, as observed in the single temperature experiments.

The growth rate during the 15°C temperature stage was similar for the experiments in which the milk was held for 24 or 48 hours at 4°C before the temperature increase. Therefore the increased storage time at 4°C, before the temperature increased to 15°C did not affect the growth rate.

4.2 GROWTH MODELS

Two secondary growth models were identified in the literature. (Lin *et al.*, 2016) fitted a square root model to experimental data for *P. fluorescens* held at constant temperatures and (Neumeyer *et al.*, 1997) tested a broth based model on milk products.

Lin et al (2016)

Lin et al. (2016) fitted a logistic model to the growth profiles at constant temperatures to determine the maximum growth rates, lag phase duration and maximum cell density at each test temperature. The maximum growth rates were found to follow the square root relationship;

$$\sqrt{k} = 0.0177\ (T + 9.15)$$

which is compared with experimental data in Figure 3. The predicted growth rates are at the lower bound of the data. It is unclear from the paper whether a single strain of *P. fluorescens* or a cocktail of strains was used.

Application of the Lin et al. (2016) growth rates to a logistic growth model, to model the two temperature experiment (4°C for 24 hours, 15°C for 6 hours), resulted in an under-prediction of growth of approximately 0.5 Log_{10} CFU/ml during the time the milk was incubated at 15°C. The growth was more consistent with the straight use of the maximum growth rate.

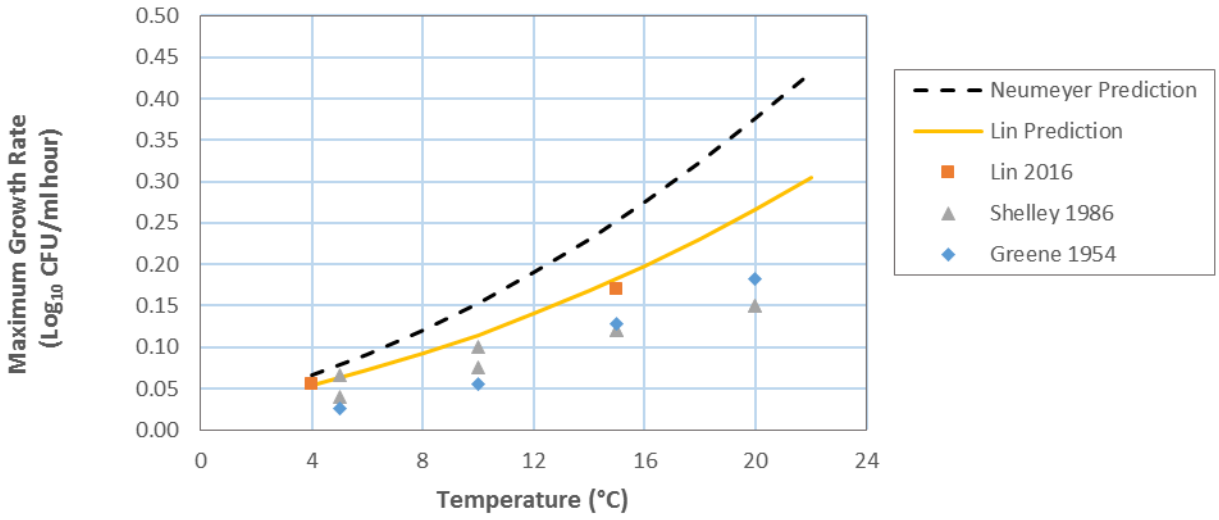


Figure 3: Comparison of *Pseudomonas* spp. maximum growth rate experimental data (heat treated milk) with model predictions.

Neumeyer et al. (1997)

The Neumeyer et al. (1997) model used a strain of *Pseudomonas* (*P. putida* 1442) that was found to have the joint highest growth rate of a number of strains that were tested in a broth matrix, with the aim of producing a worst case estimate of growth.

The broth based growth curves were fitted to a Gompertz equation and the maximum specific growth rate estimated. The maximum specific growth rate was found to relate to temperature (in the range -0.4 to 28.2°C) and water activity by the relationship;

$$\sqrt{k} = 0.1539\ (T + 7.6)\ \sqrt{a_w - 0.947}\ .$$

This relationship is plotted in Figure 3, and shows much higher growth rate estimates than the limited experimental data that have been collected. The generation times estimated by the model were compared against experimental data for raw milk with no added inoculum (T = 0-24°C). On average the model slightly over-predicted growth rates of Pseudomonads in the raw milk (bias factor 0.97 and accuracy 1.13) compared with laboratory experiments.

4.3 CONCLUSIONS

Raw milk can contain a number of different *Pseudomonas* spp. The growth rates at different temperatures depend on the milk type. The limited data located suggests *Pseudomonas* spp. will grow faster in heat treated milks than in raw milk. The data on maximum growth rates relates to a limited number of strains, and a greater variability in strain growth would be expected if more data were to be available.

The growth rate of *Pseudomonas* spp. increases with temperature over the range of 4 to 20°C. For the data that are available, maximum growth rates of 0.1, 0.17 and 0.18 Log_{10} CFU/ml hour at 10, 15 and 20°C have been observed.

The multi-temperature experiments by Lin et al. (2016), suggested limited or no growth lag existed when the milk changed from 4 to 15°C and the maximum growth rate predicted during single temperature experiments could be used to predict growth after temperature shifts from 4 to 15°C. The use of their proposed logistic model under-predicted the growth following the increase in temperature and so would not be suitable to estimate growth.

More data are needed to determine the variability of strain growth rates over this temperature range. The “worst case” broth model proposed by Neumeyer et al (1997) provides a safety buffer compared to the observed data.

5. BACILLUS CEREUS

B. cereus is one of the most important spoilage microorganisms in the dairy environment and its growth and associated enzyme production may result in a number of defects in dairy products, including milk flavour defects, rancidity, and changes in coagulation time (Kumari and Sarkar, 2016; Meer *et al.*, 1991). *B. cereus* can also cause foodborne illness through the ingestion of cells (10^5 – 10^8, (Granum, 2007)) or preformed toxins.

The natural reservoirs for *B. cereus* are decaying organic matter, fresh and marine waters, vegetables, fomites and the intestinal flora of different animals, from which soil and food products including milk and dairy products may become contaminated (Kumari and Sarkar, 2016).

B. cereus is a spore-forming bacterium and spores from some strains are heat-resistant and will survive pasteurisation (Larsen and Jorgensen, 1999).

Under ideal conditions, the minimal and optimum growth temperatures for *B. cereus* are 4°C and 30 – 40°C respectively. The optimum pH for growth is 6-7 (ICMSF, 1996).

5.1 GROWTH

5.1.1 Single temperature growth

The rate of growth of *B. cereus* in milk subjected to a constant temperature has been collected in two ways: maximum growth rates and observed growth after a given time period.

For studies which have sufficient sample time points to allow the maximum growth rate to be calculated, the data are presented in Figure 4. One study (Slovak University of Technology [STU], Combase) used heat treated milk as the substrate. For the studies of Dufeu and Leesment (1974) and Kim et al. (2013) is was unclear if raw or heat treated milk had been used.

Minimal growth was observed at temperatures up to 10°C, with growth rates increasing with temperature above 10°C. A maximum growth rate of 0.35 Log_{10} CFU/ml hour was reported at 20°C, equating to approximately a 1 log increase after 2.9 hours.

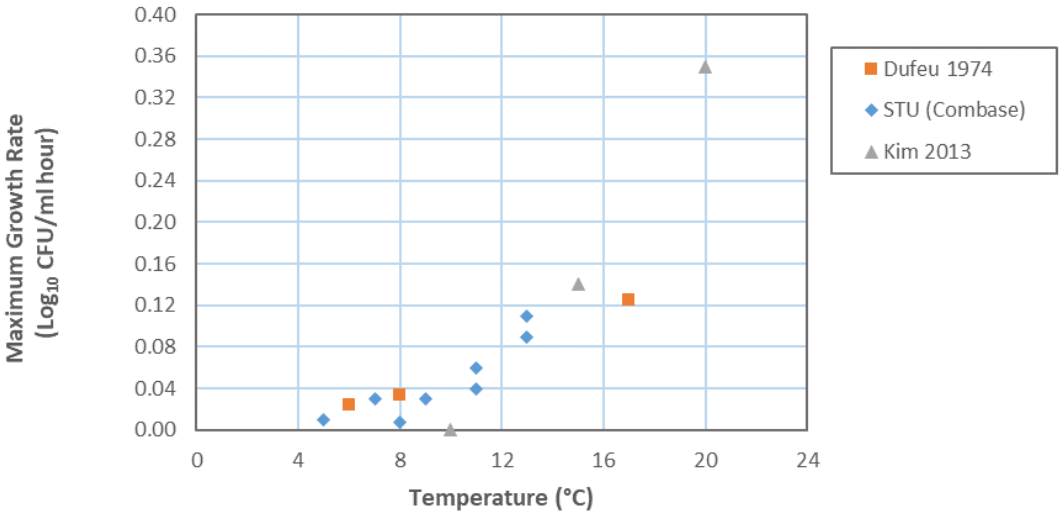


Figure 4: *B. cereus* maximum growth rates in heat treated milk from logarithmic growth phases at different constant temperatures from 6 to 20°C.

Jenson and Moir (2003) state that "*B.cereus* is generally classed as a mesophile, but psychrotropic strains are not uncommon, particularly in raw and pasteurised milk".

For the five studies that calculated the growth rate of *B. cereus*, but did not provide sufficient information to derive a maximum growth rate, the data are summarised in Appendix B Table 7. Two of the studies were performed using inoculated milk, and three were for milk with *B. cereus* already present in the milk.

Necidova *et al.* (2014) conducted experiments on pasteurised cows, goat and sheep milk, inoculated with a single strain of *B. cereus*. Less than 0.7 Log_{10} CFU/ml growth was observed after 24 hours at 8°C, and an approximately 2 Log_{10} CFU/ml growth was observed at 15°C. However, no growth was observed when the experiments were conducted using raw milk. No toxin was detected in these experiments.

In an experiment using reconstituted skim milk held at 20°C, between 0.2 and 2 Log_{10} CFU/ml growth of *B. cereus* was observed after 12 hours. After 24 hours of incubation at 20°C, 3.5 to 5 Log_{10} CFU/ml of growth was observed (Rodriquez and Barrett, 1986).

Another experiment inoculated a cocktail of 10 strains of *B. cereus* into pasteurised milk at a pH of 6.2. After 16 hours at 16°C, approximately 1 Log_{10} CFU/ml growth was observed, which increased to 1.7 Log_{10} CFU/ml after a total of 22 hours (Food Standards Agency [FSA], UK; Combase).

These results are similar, to the growth rates presented in Figure 4, and the average growth rate for the above studies at 15 - 16°C is ~0.08 Log_{10} CFU/ml.

5.1.2 Naturally contaminated milk

Two studies were found using milk that was naturally contaminated with *B. cereus*. In both studies, *B. cereus* would have been in spore form at the beginning of the experiment.

No germination of *B. cereus* was observed in freshly pasteurised milk stored at 7°C for 24 hours (Larsen and Jorgensen, 1999). Germination/growth was observed in the same milk after 4 days at 7°C.

No growth of *B. cereus* was observed after incubation of raw or matched pasteurised donkey milk at 12°C for 24 hours (Giacometti *et al.*, 2016). The *B. cereus* counts remained less than 10 CFU/ml during this time. *B. cereus* was present in the samples, given a count of 2.9 Log_{10} CFU/ml in the pasteurised milk after 3 days. No growth was observed in the raw milk after 3 days. The heat-shocking of spores during pasteurisation may promote the subsequent germination of the spores.

5.2 GROWTH MODELS

Kim et al (2013)

A predictive model based on the Gompertz function (Appendix C.2) was fitted to *B. cereus* (strain KCCM40935) growth curves from experiments conducted at 15, 20 and 30°C in an undefined retail milk (Kim *et al.*, 2013). No growth was detected at 10°C after 48 hours. The maximum growth rate was found to be related to temperature via the function;

$$\sqrt{\mu_{max}} = 0.0331(T - 3.4108)$$

The predicted maximum growth rate by temperature compared to experimental data is shown in Figure 5. The solid line represents the growth predictions in the range of the three

temperatures the model was based on. The dashed line represents an extrapolation of the model to lower temperatures.

The model provides a reasonable fit to the data provided, however experiments in raw milk, over the temperature range of 10 to 20°C and for a variety of *B. cereus* strains would be required to confirm the usefulness of the model.

Zwietering et al (1996)

Zwietering *et al.* (1996) proposed the use of an exponential growth model with a lag phase, for a primary growth model. The maximum growth rate was calculated using a cardinal model with pH, water activity and a squared temperature component. For the pasteurised milk matrix, they suggested the following relationship between temperature and growth rate;

$\sqrt{k} = 0.0354(T - T_{min})$ where $T_{min} = 0.$

The model was tested against *B. cereus* growth in freshly pasteurised milk, incubated at temperatures of 6 to 12°C. However, the first samples at 12°C were taken after 48 hours and the *B. cereus* concentration at the start of incubation was not measured. The enumeration method included incubation of plates at 30°C, so only mesophilic strains may have been counted. It is also not clear from the paper what the units of density are, for the calculations below will assume a cell density of CFU/ml.

After 48 hours at 12°C, the concentration in the cartons ranged from 1.5 to 3.5 Log_{10} CFU. It is unclear if this variation is due to the variations in initial concentrations and strains between the cartons, or variation in growth rates, or a combination of the two. Therefore it is difficult to evaluate the model compared to the experimental data presented. The first sampling time points for the lower temperatures were much greater than 24 hours, and showed a similar variation in growth (2-3 Log_{10} CFU).

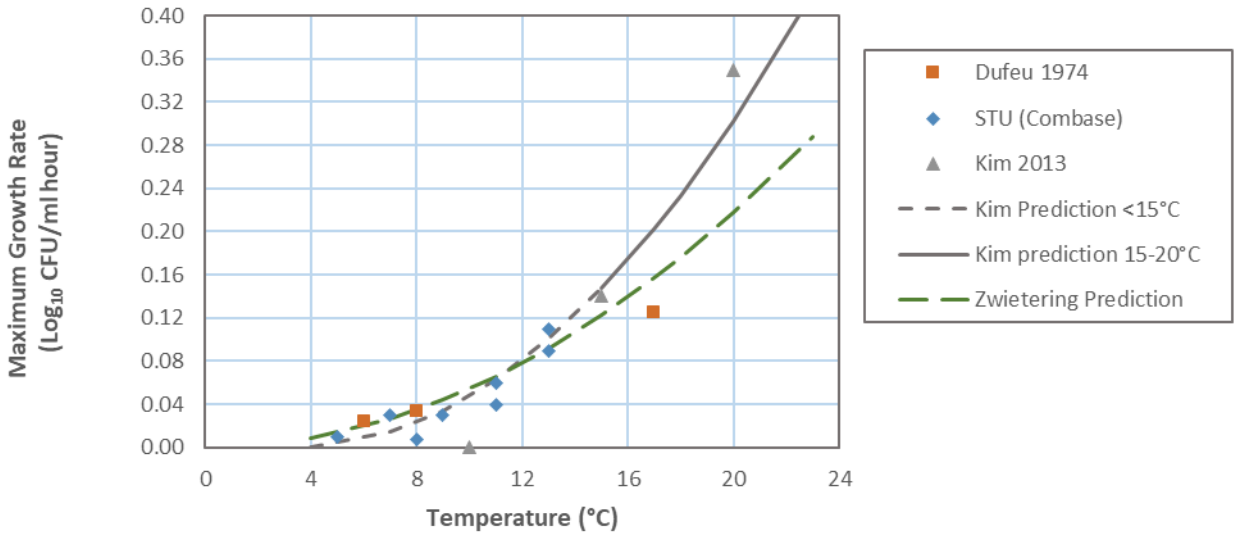


Figure 5: Maximum growth rate experimental data and Kim et al. model prediction for *B. cereus* in milk

5.3 CONCLUSIONS

The *B. cereus* species includes a range of strains with differing abilities to grow and sporulate at different temperatures.

There are limited data available to describe the growth of *B. cereus* in raw milk or evaluate growth models in the temperature range 4 to 20°C. From the available data for raw and heat treated milk, there appears to be very limited growth at temperatures below 10°C, with expected growth below 1 Log_{10} CFU/ml in 24 hours.

Above 10°C, the rate of growth increases with temperature, with a 5 Log_{10} CFU/ml increase observed over 24 hours at 20°C in reconstituted milk or an unknown retail milk. However, this was limited by the cell population reaching close to stationary phase populations before the end of the incubation period. The maximum growth rate would estimate a ~8 log increase in 24 hours.

The effect of the milk type (e.g. raw vs heat treated) on the expected growth rates is not known; only one study was confirmed to have tested raw milk, and for this study no growth was observed at incubation temperatures of 8 and 15°C.

6. *CAMPYLOBACTER* SPP.

Campylobacter jejuni is found in the intestinal tract, udder, and faeces of cattle, in poultry and wild birds, and in contaminated water sources. *Campylobacter* spp. may be shed directly into the milk when the animal has clinical or subclinical mastitis due to *Campylobacter* infection, or indirectly through faecal contamination (Orr *et al.*, 1995).

The prevalence of *C. jejuni* in 25ml samples of bulk tank milk in New Zealand investigated in two studies was 1 out of 296 samples (0.3%) and 0.6% (CI: 0.07, 2.1), respectively (Hill *et al.*, 2012; Marshall *et al.*, 2016).

Campylobacter spp. do not multiply at temperatures below 30ºC (Park, 2002). Although unable to grow below 30ºC, they remain metabolically active, and are motile at temperatures as low as 4ºC (Park, 2002).

6.1 GROWTH DATA

Growth of *Campylobacter* spp. populations was not observed in experimental studies in raw or heat treated milk incubated in the temperature range 4 to 20°C (Christopher *et al.*, 1982; Doyle and Roman, 1982; Simms and Rae, 1989). These papers show that *Campylobacter* spp. may initially survive, but will then steadily decline over several days.

One study by Barrell (Barrell, 1981) plotted slight growth of one strain (2658) of *Campylobacter* spp. at 4 and 21°C in the first six hours of incubation in raw milk. The other six strains showed a gradual decline in culturable population size. *Campylobacter* spp. are not expected to grow at these temperatures. The author suggested the population increase could have been due to “an acclimatisation of the organism to the milk”. It also remains possible that another species present in the raw milk may have been mistakenly enumerated for *Campylobacter,* even though a selective enumeration procedure and morphological conformation was used.

6.2 CONCLUSION

Campylobacter spp. are not expected to grow in raw or heat treated milk in the temperature range of 4 to 20°C.

7. *CLOSTRIDIUM* SPP.

Clostridia are thermoduric (survive pasteurisation) spore-forming organisms that can cause craters and cracks in certain cheeses due to gas production. Five hundred spores per litre of milk are sufficient to cause a late gas defect.

Clostridia are found in the intestinal tract of humans and animals, as well as in the environment and silage. *Cl. perfringens* may also be involved in bovine mastitis so can be part of the flora of raw milk (Gilmour and Rowe, 1990).

Clostridium botulinum and *Clostridium perfringens* are also organisms of concern as causes of foodborne illness.

7.1 *Clostridium botulinum*

Cl. botulinum is the cause of foodborne botulism, caused by the ingestion of foods (including milk) containing botulinum neurotoxin types (BoNTs) A, B, E and on rare occasions F. The heat stability of BoNT-A and BoNT-B at conventional milk pasteurisation temperatures (63 °C, 30 min) depends on the serotype; BoNT serotype A and E is inactivated but serotype B is heat-stable in milk and not inactivated by pasteurisation (Rasooly and Do, 2010).

Group I strains produce A, B and F toxins, and have minimum and optimum growth temperatures of 10°C and 35 – 40°C respectively. Group II strains which produce B, E and F toxins have minimum and optimum growth temperatures of 3°C and 25 - 30°C, respectively, when all other growth conditions are optimal.

Read *et al*. (1970) conducted experiments to determine the growth and BoNT-E production of 6 strains of *Cl. botulinum* inoculated into sterile milk at incubation temperatures from 4.4 to 20°C. The starting inoculum was ~10^3 heat-shocked spores per ml.

Table 1: Summary of the results of growth experiments of *Cl. Botulinum* in sterile milk (Read et al. 1970)

Temperature (°C)	Cell Log Growth of Tenno strain (Log_{10} CFU/ml)	Time for first detection of toxin	Lowest cell concentration from 6 strains at the time of first detection of (Log_{10} CFU/ml)
4.4	No growth	Not detected	Toxin not detected, maximum cell concentration after 100 days was 5 Log_{10} CFU/ml
7.2	~ 0.5 after a week	70 days	5.1
10	~ 1.0 after a week	28 days	4.5
15	~ 2.0 after a week	14 days	4.3
20	~ 1.0 after a day	3 days	4.6

The Tenno strain of *Cl. botulinum* grew at 7.2°C, but the first time point with a detectable level of toxin was at 70 days. The other strains did not grow significantly at 7.2°C according to the paper. The results from Read *et al* (1970) suggest that for the strains considered, the growth of *Cl. botulinum* would be slow at temperatures below 20°C, with no more than 1 Log_{10} CFU/ml growth after 24 hours

In the temperature range of 10 to 20°C, the concentration of cells present at the time of toxin detection was in the range of 4.3 to 6 Log_{10} CFU/ml

7.2 *Clostridium perfringens*

The ingestion of Types A and C *Cl. perfringens* cells is associated with foodborne illness, with type C causing more severe illness. The cells which survive passage through the stomach can sporulate in the small intestine, releasing a toxin which causes illness (Bates and Bodnaruk, 2003).

The optimal temperature for growth is 43 to 45°C. The minimum temperature for growth is 6°C, but growth is considered to be very slow below 15°C (McClane, 2007).

No data were found relating to *Cl. perfringens* growth in milk in the temperature range of 4 to 20°C.

7.3 *Clostridium butyricum*

Cl. butyricum is associated with late blowing of cheeses, approximately 1-2 months after manufacture. “The optimum temperature for growth is 30-37°C, but many strains grow equally well at 25°C and some at 10°C” (Gilmour and Rowe, 1990).

8. *ESCHERICHIA COLI*

Most strains of *E. coli* are considered harmless and are part of the normal intestinal microflora of people and animals. However, the species also includes certain strains that can cause severe illness in humans. *E. coli* can be a cause of mastitis in milking animals (Bramley and McKinnon, 1990).

The prevalence of *E. coli* O157 in 25ml samples of bulk tank milk in New Zealand investigated in two studies was 1.0% and 0.6% (CI: 0.07, 2.1) (Hill *et al.*, 2012; Marshall *et al.*, 2016).

In broth, *E. coli* can grow at temperatures in the range of 7 to 46°C, with an optimum growth temperature range of 35 to 40°C. The optimum pH for growth is in the range of 6 to 7 (ICMSF, 1996).

8.1 GROWTH

8.1.1 Single temperature data

The rate of growth of *E.coli* in milk subjected to a constant temperature has been collected in two ways: maximum growth rates and observed growth after a given time period.

For studies which have sufficient sample time points to allow the maximum growth rate to be calculated, the data are presented in Figure 6. The three studies (Acai *et al.*, 2016; Kasrazadeh and Genigeorgis, 1995; Kauppi *et al.*, 1996) all used heat-treated milk as the substrate. Few experiments were conducted with temperatures between 12 and 20°C. Minimal growth was observed at temperatures up to 20°C, with a maximum growth rate of 0.2 Log_{10} CFU/ml hour reported at 20°C, equating to approximately a 1 log increase after 5 hours.

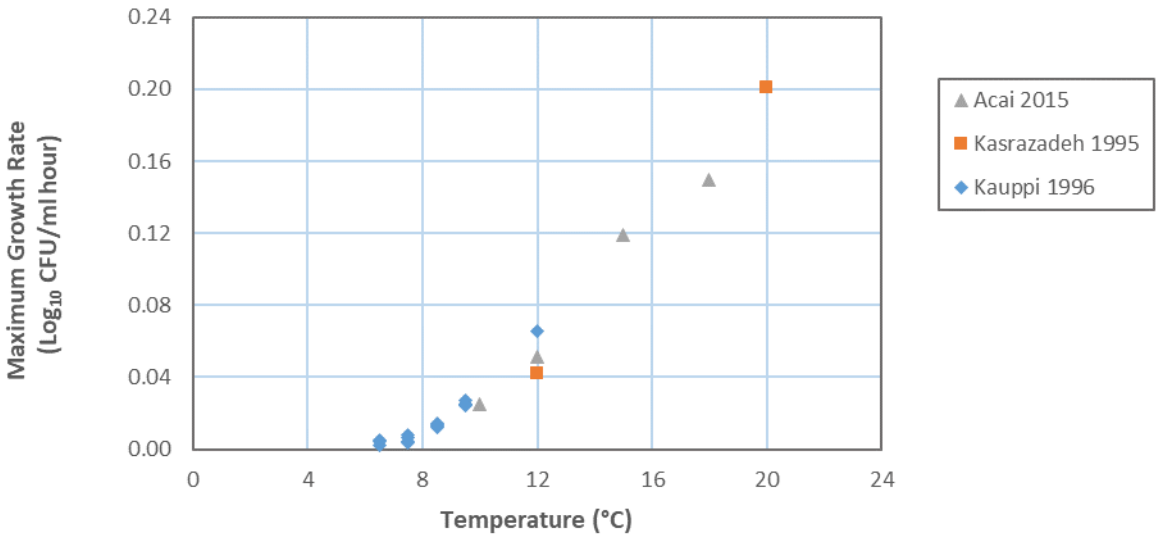


Figure 6: *E. coli* maximum growth rates in heat treated milk from logarithmic growth phases at different constant temperatures from 6 to 20°C.

For studies that enumerated the growth of *E. coli*, but did not provide sufficient information to derive a maximum growth rate, the data are summarised in Appendix B Table 8.

These studies consistently demonstrated no growth, at temperatures in the range 4 to 8°C, 24 hours after inoculation in raw or heat-treated cow's milk. One study (Zapico *et al.*, 1995) using raw goat's milk inoculated with goat milk isolates of *E. coli,* recorded slight growth of *E. coli* (~0.6 Log_{10} CFU/ml) after 24 hours at 8°C.

At an incubation temperature of 15°C, one raw milk study showed no growth after 16 hours (Carminati *et al.*, 2008), while another recorded growth of approximately 1.5 Log_{10} CFU/ml after 24 hours (Wang *et al.*, 1997). For an incubation temperature of 20°C, between 1 and 3 Log_{10} CFU/ml growth of *E. coli* was observed in raw or heat treated milk after 24 hours (Alhelfi *et al.*, 2012; Carminati *et al.*, 2008; Mamani *et al.*, 2003; Zarei *et al.*, 2010). This is less growth than would be predicted by using the maximum growth rate estimated by Kasrazadeh and Genigeorgis (1995); 4.8 Log_{10} CFU/ml.

For studies presented in this section there is no clear difference in growth rates between pathogenic and non-pathogenic strain types. In a study by Salter et al (1998) the growth rates of faster growing pathogenic strains and a faster growing non-pathogenic strain (M23) were found to be similar.

8.1.2 Naturally contaminated milk

No studies were found which investigated the growth of *E. coli* in raw milk, involving no added inoculum.

8.1.3 Multi temperature data

Giacometti et al. (2012) conducted growth experiments simulating worst case farm to consumption conditions, determined from a survey conducted with domestic raw milk consumers who bought milk from off-farm vending machines. The growth of *E. coli* O157:H7 in raw milk at different stages in the storage chain is summarised in Table 2. After the four days duration of this experiment just under 2 Log_{10} CFU/ml of growth had occurred. Most growth occurred over the extended periods at 11-12°C, during storage in the vending machine and in the home.

Table 2: Growth of *E. coli* O157:H7 in raw milk during the farm to consumption chain for raw milk sold via a vending machine (Giacometti *et al.*, 2012).

Phase	Duration (hours)	Temperature (°C)	Population count (mean±SD Log_{10} CFU/ml)
Inoculation	0	-	2.14±0.02
Transport to vending machine	5	7	2.28±0.14
Storage in vending machine	22.5	11	3.15±0.30
Transport home	0.5	30	2.90±0.02
Home storage	68	12	3.97±0.28

8.2 GROWTH MODELS

Salter et al (1998)

An extended square root model was derived for *E. coli* strain M23 in a nutrient broth (Oxoid CM67) and tested against milk types (Salter *et al.*, 1998). The model was designed to predict the maximum growth likely to be observed from *E. coli* strains. However, there is a discrepancy in the paper between the model presented and the predicted generation times for media and food types.

Ačai et al (2016)

Ačai et al (2016) fitted a Baranyi and Roberts model (Appendix C.2) to the growth of *E. coli* strain BR in UHT milk incubated at temperatures between 10 and 37°C. The maximum specific growth rate, k, was estimated for each temperature. The resulting specific growth rates were found to be related to the incubation temperature by the square root relationship:

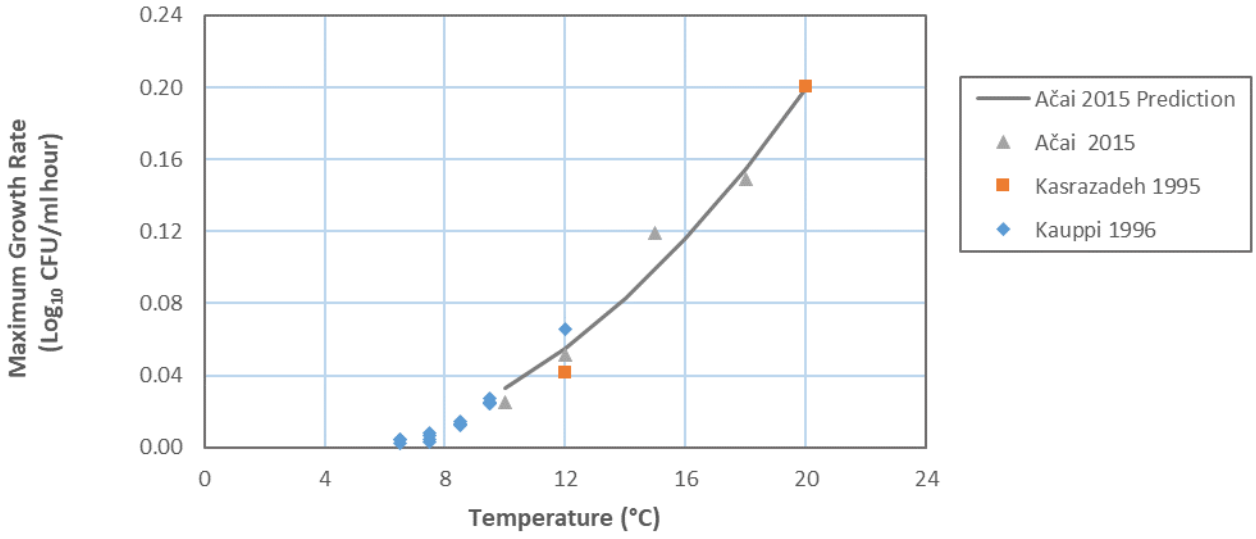
$$\sqrt{k} = b(T - T_{min}) \quad \text{where } b = 0.024\ (\text{SE}.0026) \text{ and } T_{min} = 3.10\ (\text{SE}^2{:}1.39)\ .$$

The authors developed a further model which predicted the simultaneous growth of *E. coli* and LAB (commercial starter culture) over time, when both are inoculated into UHT milk. The model accounts for the possible competition between *E. coli* and LAB. Their experimental results could be modelled using the competition model based on coupled Baranyi and Roberts type models for *E. coli* and LAB and interaction/ competition terms.

These models are based on experiments using a single strain of *E. coli* and the experimental set up was the same for calculating the maximum growth rates and the competition terms. While such a model may prove useful for product development, the variability in types of microorganisms present in raw milk, make such models difficult to use for generic guidance on growth of *E. coli* in raw milk.

The maximum growth rate model proposed by Ačai *et al* (2016) is shown along with the data from Kasrazadeh and Genigeorgis (1995) and Kauppi *et al* 1996) (

Figure 7). However, further experimental data, including raw milk experiments, would be



needed to validate the model.

[2] SE: Standard error of parameter estimate.

Figure 7: Maximum growth rate experimental data and Ačai *et al* (2016) model prediction for *E. coli* in heat treated milk.

8.3 CONCLUSIONS

There are limited data available to describe the growth of *E. coli* in raw milk in the temperature range 4 to 20°C. From the data on raw and heat treated milk that are available, there appears to be little growth at temperatures below 10°C, with expected growth below 1 Log_{10} CFU/ml in 24 hours.

Above 10°C, the rate of growth increases with temperature, with 1 to 3 Log_{10} CFU/ml increase observed over 24 hours at 20°C in raw milk. This is less growth than predicted using the observed maximum growth rate at 20°C in heat-treated milk, which predicts up to 4.8 Log_{10} CFU/ml increase in cell concentration.

There are currently no growth models based on raw milk data. The Ačai *et al* (2016) model for maximum specific growth rate as a function of temperature fits the limited data set well, but further data, including raw milk data would be needed to validate the model for a variety of *E. coli* strains.

9. *LISTERIA MONOCYTOGENES*

Listeria monocytogenes is found in soil and water and has been isolated from many environmental sources. Thus, *L. monocytogenes* is ubiquitous in the dairy environment and may contaminate milk during milking and can also be a cause of mastitis in milking animals resulting in direct shedding into raw milk.

In broth *L. monocytogenes* can grow at temperatures in the range -0.4 to 45°C, with an optimum growth temperature of 37°C. The optimum pH for growth is 7 (ICMSF, 1996).

9.1 GROWTH DATA

9.1.1 Single Temperature Data

The rate of growth of *L. monocytogenes* in milk subjected to constant temperatures has been collected in two ways; maximum growth rates and observed growth after a given time period.

For studies which have sufficient sample time points to allow the maximum growth rate to be calculated, the data are presented in Figure 8 (Alavi *et al.*, 1999; Buchanan and Klawitter, 1991; Pearson and Marth, 1990; Rajkowski *et al.*, 1994; Rosenow and Marth, 1987). Only one study by Farber et al (1990) was conducted using raw milk. Minimal growth was observed at temperatures below 20°C, with a maximum growth rate of 0.18 Log_{10} CFU/ml hour reported at 19°C, equating to approximately a 1 log increase after 5-6 hours.

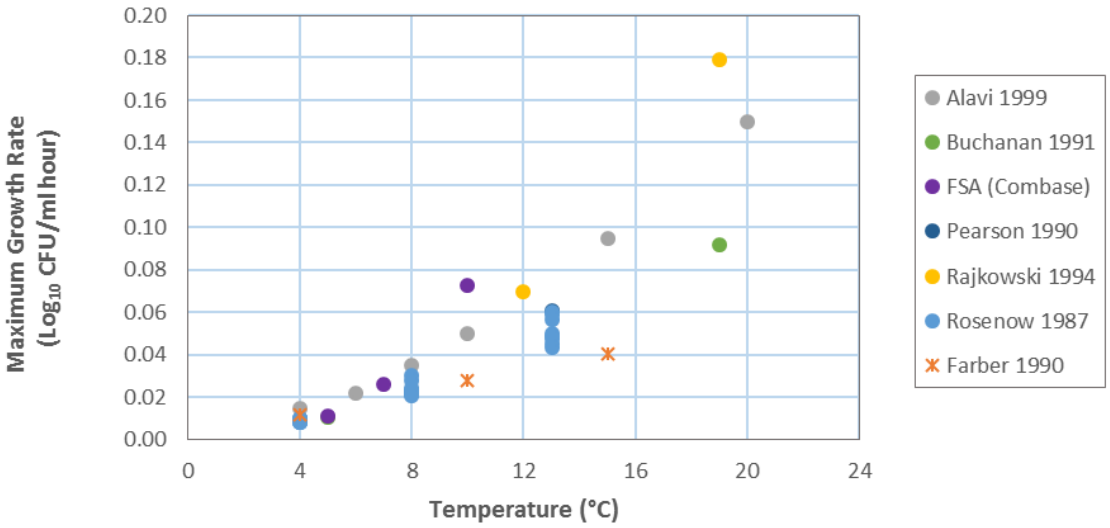


Figure 8: *L. monocytogenes* growth rates in milk from logarithmic growth phases at different constant temperatures from 4 to 20°C, filled circles represent heat treated milk and * represents raw milk data.

For studies that enumerated the growth of *L. monocytogenes*, but did not provide sufficient information to derive a maximum growth rate, the data are summarised in Appendix B Table 9. At temperatures in the range of 4 to 15°C, the maximum growth observed in raw milk was 0.5 Log_{10} CFU/ml after 24 hours (Carminati *et al.*, 2008; Gaya *et al.*, 1991; Marshall and Schmidt, 1988; Rodriguez *et al.*, 1997; Zapico *et al.*, 1993).

In contrast, greater growth was observed in heat treated milk, with up to 3 Log_{10} CFU/ml of growth in 24 hours (Denis and Ramet, 1989; Donnelly and Briggs, 1986; Kamau *et al.*, 1990). At 20°C, between 0.6 and 2.3 Log_{10} CFU/ml growth in raw milk was observed after 24 hours (Carminati *et al.*, 2008; Zapico *et al.*, 1993). These data are consistent with the lower bound of growth rates presented in Figure 8.

9.1.2 Naturally contaminated milk

Farber et al. (1990) collected milk from a cow which was known to shed *Listeria monocytogenes* at levels of 3 to 4 CFU/ml. The freshly collected milk was stored aerobically at 4, 10 and 15°C and *L. monocytogenes* enumeration was completed at the initial time point and then daily. At a temperature of 4°C, a three to five day lag phase was observed before growth, and at 10°C, the lag phase lasted for at least 24 hours. The growth rate at all three temperatures was below 0.05 log_{10} CFU/ml hour, although the maximum growth rate did increase with increasing storage temperature.

9.1.3 Multiple Temperature Data

Giacometti et al. (2012) conducted growth experiments simulating worst case conditions from farm to consumption, based on data from a survey conducted with domestic raw milk consumers who bought milk from off-farm vending machines. The growth of *L. monocytogenes* in raw milk at different stages in the storage chain is summarised in Table 3. After the four days duration of this experiment approximately 1 Log_{10} CFU/ml of growth had occurred. Growth mainly occurred during the long period of home storage.

Table 3: Growth of *L. monocytogenes* in raw milk during the farm to consumption chain for raw milk sold via a vending machine (Giacometti *et al.*, 2012).

Phase	Duration (hours)	Temperature (°C)	Population count (mean±SD Log_{10} CFU/ml)
Inoculation	0	-	2.18±0.03
Transport to vending machine	5	7	2.30±0.11
Storage in vending machine	22.5	11	2.45±0.24
Transport home	0.5	30	2.50±0.13
Home storage	68	12	3.25±0.31

Xanthiakos et al. (2006) conducted experiments in pasteurised milk, under periodically changing temperatures. *L. monocytogenes* Scott A concentration increased by less than 1 Log_{10} CFU/ml, during a period of storage of 12 hours at 4°C followed by 12 hours at 12°C. In another experiment, *L. monocytogenes* Scott A concentration increased by approximately 1.3 Log_{10} CFU/ml during 28 hours of storage, comprising 8 hours at 10°C, 12 hours at 16°C and 8 hours at 6°C.

9.2 GROWTH MODELS

Augustin et al. (2005)

Augustin et al. (2005) derived a cardinal model to predict the maximum specific growth rate of *L. monocytogenes* in liquid dairy products. The simplified version of the model (excluding inhibitory substances), is a function of temperature (T), pH and water activity (aW), as well as their cardinal values of minimum, maximum and optimum values for growth:

$k_{max} = k_{opt}\ \tau(T)\ \rho(pH)\ \alpha(aW)\ \xi(T, pH, aW)$,

where ξ is a multiplier between 0 and 1, which takes into account interactions between the environmental factors and the growth rate. For milk in the temperature range of 4 to 20°C this factor was evaluated to be 1 (no interaction effects).

The liquid dairy parameter value for k_{opt}, the optimal specific growth rate, was derived from 14 different studies using liquid dairy growth media. The minimum cardinal values of T, pH and aW were fitted to data from a range of studies, including growth of *L. monocytogenes* in microbiological media, cheese, meat and seafood products as well as the dairy products.

Augustin et al. (2005) recognised the variability in growth rates between studies and strains, so produced an upper and lower bound on the expected growth rates. The predicted and upper bound are given in Figure 9.

Xanthiakos et al. (2006)

Xanthiakos et al. (2006) fitted a Baranyi and Roberts model (Appendix C.2) to the growth of *L. monocytogenes* Scott A in pasteurised milk incubated at 4, 8, 12 and 16°C. The maximum specific growth rate, k, was estimated for each temperature. The resulting specific growth rates were found to be related to the incubation temperature by the square root relationship:

$\sqrt{k} = b(T - T_{min})$ where $b = 0.024$ and $T_{min} = -2.32$.

The estimated growth rates predicted by this model are shown in Figure 9.

Numerical integration of the Baranyi and Roberts model (Appendix C.2, m=1, N_{max}=$10^{8.5}$) to predict the growth during dynamic storage temperatures gave predictions within 1 Log_{10} CFU/ml of observed experimental concentrations. These experiments used a single strain of *L. monocytogenes* and the constant and dynamic temperature experiments used the same experimental procedures, apart from incubator settings, providing best case scenario for testing models.

Murphy et al. (1996)

A predictive model based on the Gompertz function (Appendix C.2) was fitted to *L. monocytogenes* (strain NCTC 5348) growth curves from experiments conducted at temperatures between 3 and 35°C in reconstituted skim milk (Murphy *et al.*, 1996). The natural logarithm of the Gompertz parameters B, C and M were fitted to a cubic equations of temperature, pH and NaCl. The maximum growth rate by temperature is shown in Figure 9.

The results were then compared to the growth rate from experiments in pasteurised and UHT milk held at 6 and 9 °C. The model slightly over-predicted the maximum growth rates compared to the observed growth, however the predicted maximum growth rate was only 0.043 Log_{10} CFU/ml hour. The model was not validated at temperatures over 9°C in milk and was only developed and tested for a single strain of *L. monocytogenes*.

Alavi et al. (1999)

Alavi et al. (1999) fitted a Baranyi and Roberts model (Appendix C.2) to the growth of *L. monocytogenes* Scott A in sterilised whole milk incubated at 4, 6, 8, 10, 15 and 20°C. The maximum specific growth rate, k, was estimated for each temperature. The resulting specific growth rates were fitted to an extended square root relationship for incubation temperature:

$\mu_{max} = [0.0185(T + 1.73)]^2 \times \left[1 - e^{0.1392(T-44.81)}\right]$.

The estimated growth rates predicted for this model are shown in Figure 9.

Numerical integration of the Baranyi and Roberts model (Appendix C.2, m=1, N_{max}=$10^{7.47}$) to predict the growth during dynamic storage temperatures gave predictions within 1 Log_{10} CFU/ml of observed experimental concentrations. The fit of the model was dependent on the choice of the model parameter for cell adaption (α). These experiments used a single strain of *L. monocytogenes,* Scott A, inoculated into sterilised milk held in an incubator fluctuating between 10 and 20°C at a rate of ±5°C/hour.

9.3 COMPARISON OF GROWTH RATES TO DATA

Figure 9 shows a comparison of the maximum growth rate data presented in Figure 8 to the growth models described in the previous section of this report. All models follow the general trend of the limited milk maximum growth rate data. The upper bound of the Augustin model covers the upper bound of the data. This would be expected, as the upper and lower bounds of the model were chosen to cover 95% of the data from the 14 studies used to develop the liquid dairy model.

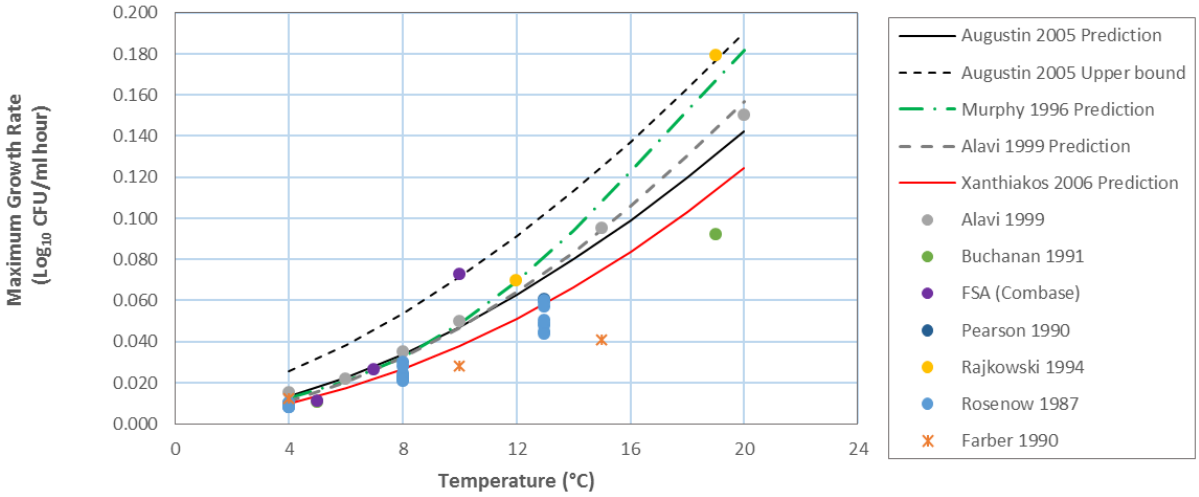


Figure 9: Maximum growth rate experimental data and model predictions for *L. monocytogenes* in milk, raw milk data is represented by *.

9.4 CONCLUSIONS

A number of data and modelling studies have been conducted for *L. monocytogenes* in milk, however only one raw milk study provided enough data to calculate maximum growth rates in raw milk. The raw milk growth rates were less than the rates reported for heat treated milk.

The maximum growth data combined with data from studies with insufficient data to calculate a maximum growth rate, suggest that the growth of *L. monocytogenes* in raw milk would be very slow at temperatures up to 12-15°C, with less than 1 Log growth in 24 hours. At 20°C, up to 3 Log of growth in 24 hours of *L. monocytogenes* was recorded in raw milk. However this is based on a small number of data points

Growth of *L. monocytogenes* in heat treated milk at temperatures of 10 to 15°C was greater than raw milk with up to 3 Log growth after 24 hours.

A number of maximum growth rate models have been proposed which fall within the range of the experimental data points collected, however all but one study is based on single strains of *L. monocytogenes*.

Application of Gompertz or Baranyi and Roberts growth models for heat treated milk undergoing dynamic temperature incubation, has shown good agreement between model predictions and experimental results. However, this comparison can be considered best-case, as the experimental set up and strain used were the same as those used to establish the model parameters.

10. *SALMONELLA* SPP.

The primary reservoir of *Salmonella* is the intestinal tract of both warm and cold-blooded vertebrates, with many animal species being asymptomatic carriers. Infected animals can shed large numbers of *Salmonella* cells in their faeces, leading to contamination of hides, fleece, and skin, and the surrounding environment including soil, pasture, streams and lakes (FSANZ, 2009).

No *Salmonella* was detected in 25ml samples of bulk tank milk in New Zealand investigated in two independent studies (n=297, n=400) (Hill *et al.*, 2012; Marshall *et al.*, 2016).

The temperature range for growth of *Salmonella* spp. in broth is 5.2 - 46.2°C, with the optimal temperature being 35 - 43°C. The optimum pH for growth is 7 to 7.5 (ICMSF, 1996).

10.1 GROWTH

10.1.1 Single Temperature Data

No growth of *Salmonella* was observed in pasteurised milk (Bao *et al.*, 2015; Kasrazadeh and Genigeorgis, 1994) or raw milk (Giacometti *et al.*, 2012) at 4 to 8°C.

Only two studies (Bovill *et al.*, 2000; Kasrazadeh and Genigeorgis, 1994) provided maximum growth rate estimates for *Salmonella* spp. above 8°C; the rates are shown in Figure 10. Both of these studies were performed using pasteurised milk.

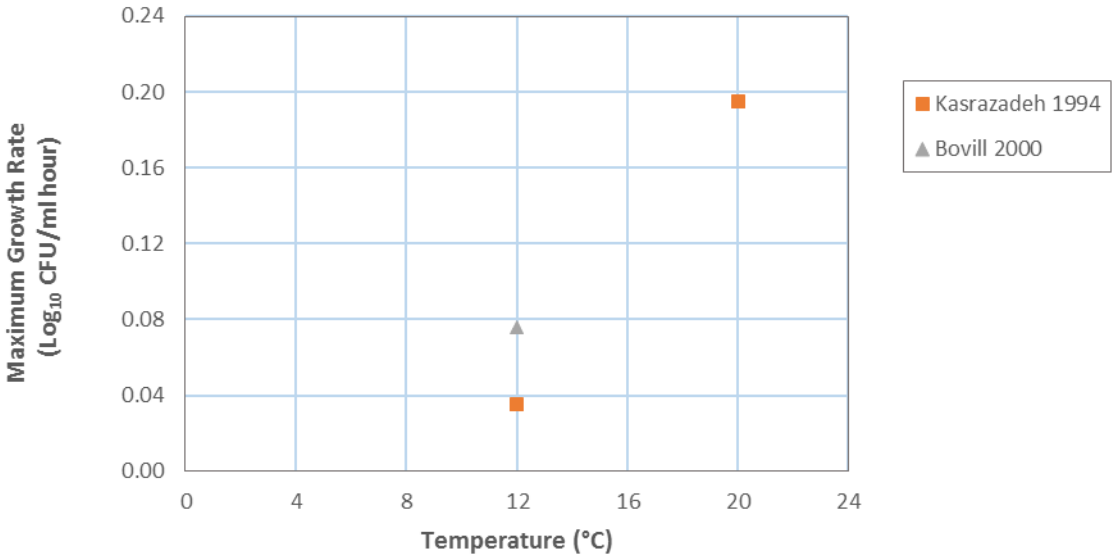


Figure 10: *Salmonella* maximum growth rates in heat treated milk from logarithmic growth phases at different constant temperatures from 12 to 20°C.

A study by Carminati et al. (2008) examined pathogen growth during the creaming of raw milk, by incubating inoculated fresh raw milk in 4L canisters at 8, 15 and 20°C for 16 hours. No growth of *Salmonella* was detected in whole milk after incubation at 8°C, with little growth (no quantification was given) detected after incubation at 15°C.

Incubation at 20°C resulted in the growth of *Salmonella* spp. with an approximately 1 Log_{10} CFU/ml increase in the skim milk, and approximately 3 Log_{10} CFU/ml increase in the cream compared to the original whole milk. The greater concentration of *Salmonella* cells in the cream is to be expected. *Salmonella* cells adhere to the membrane of fat globules, which rise through the liquid milk to form the cream layer, transporting the *Salmonella* cells with them.

10.1.2 Naturally contaminated milk

No studies were found which investigated the growth of *Salmonella* spp. in raw milk, involving no added inoculum.

10.1.3 Multiple temperature data

Giacometti et al. (2012) conducted growth experiments simulating worst case farm to consumption conditions defined from a survey conducted with domestic raw milk consumers who bought milk from vending machines. The growth of *S.* Typhimurium in raw milk at different stages in the storage chain is summarised in Table 4. After the four days duration of this experiment approximately 1.3 log of growth had occurred.

Table 4: Growth of *S.* Typhimurium in raw milk during the farm to consumption chain for raw milk sold via a vending machine (Giacometti *et al.*, 2012).

Phase	Duration (hours)	Temperature (°C)	Population count (mean±SD Log_{10} CFU/ml)
Inoculation	0	-	1.88±0.09
Transport to vending machine	5	7	1.94±0.10
Storage in vending machine	22.5	11	2.00±0.01
Transport home	0.5	30	2.72±0.24
Home storage	68	12	3.20±0.06

10.2 GROWTH MODELS

Kazrazadeh and Genigeorgis (1994) suggested a square root relationship for the specific maximum growth rate of *Salmonella* in milk for the temperature range 12 to 30°C. Fitting a linear function to their data gives the following function

$$\sqrt{k} = b(T - T_{min}) \quad \text{where} \quad b = 0.0375 \quad \text{and} \quad T_{min} = 3.56\,℃ \quad \text{for} \quad 12 \leq T \leq 30℃$$

This relationship is only based on three data points (12, 20 and 30°C) representing the growth of a pool of three strains of *S.* Typhimurium in pasteurised milk.

10.3 CONCLUSIONS

There are minimal data available to describe the maximum growth rate of *Salmonella* spp. in milk products over the temperature range of 4 to 20°C, with no maximum growth rate data available for raw cow's milk.

A single square root model for *S.* Typhimurium has been proposed, however this is based on only three data points and there are insufficient data to evaluate if this relationship could be used for a more generic model.

11. *STAPHYLOCOCCUS AUREUS*

Staphylococcus aureus is ubiquitous and inhabits the mucous membranes and skin of most warm-blooded animals, including all food animals and humans[3]. S. aureus is a recognised cause of mastitis in dairy herds (Bramley and McKinnon, 1990).

S. aureus was detected in 25ml of bulk tank milk in New Zealand was found to vary, with 21% of samples with no detection, 60% of samples *S. aureus* counts were >1 but <100 cfu/ml and one sample (0.34%) had counts $>10^4$ cfu/ml (Hill *et al.*, 2012). It is recognised that *S. aureus* concentration needs to exceed 10^5 cfu/ml for production of sufficient toxin to cause human illness.

S. aureus is readily killed at cooking and pasteurisation temperatures. In contrast, *S. aureus* enterotoxins are extremely heat resistant. Heat resistance for enterotoxin B has been reported at D_{149} = 100 min (a_w of 0.99) (Paulin *et al.,* 2001). *S. aureus* enterotoxins were reduced, but still detectable, after pasteurisation for 15 sec at 72ºC, 85ºC and 92ºC (87%, 52% and 45% of samples, respectively) (Necidova *et al.*, 2014).

The temperature range for growth of *S. aureus* is 7 - 48°C with optimum growth occurring at 35 - 40ºC, the optimum pH for growth is in the range 6-7. The temperature range for toxin production is 10 - 48ºC with the optimum temperature range of 40 - 45ºC (Bergdoll and Lee Wong, 2006; Paulin *et al.*, 2012).

11.1 GROWTH

11.1.1 Single temperature data

The rate of growth of *S. aureus* in milk subjected to constant temperatures has been collected in two ways: maximum growth rates and observed growth after a given time period.

For studies which have sufficient sample time points to allow the maximum growth rate to be calculated, the data are presented in Figure 11. Four studies considered the growth of single strains of *S. aureus* in heat treated milk (Fujikawa and Morozumi, 2006; Kamau *et al.*, 1990; Rajkowski *et al.*, 1994), with one set of data submitted to Combase by the University of Tasmania (UTAS). It is not clear from the Combase description, as to why the UTAS, *S. aureus* maximum growth rates at 17.5°C are so variable.

The Medvedova *et al.* (2009) study considered the growth of three different strains in heat treated milk and the Kim *et al.* (2013) study considered a single strain of S. aureus in an undefined retail milk type.

Minimal growth was observed at temperatures up to 10°C, with a maximum growth rate of below 0.04 Log_{10} CFU/ml hour reported. The growth rate increases with temperature, with a growth rate of 0.24 Log_{10} CFU/ml hour at 21°C, equating to a 1 log increase after approximately 4 hours.

For studies that enumerated the growth of *S. aureus*, but did not provide sufficient information to derive a maximum growth rate, the data are summarised in Appendix B Table 10. No growth of *S. aureus* was observed in raw or heat treated milks over 16-24 hours at temperatures up to 10°C (Carminati *et al.*, 2008; Donnelly *et al.*, 1968; Valihrach *et al.*, 2013; Zhang *et al.*, 2008).

[3] US Centers for Disease Control and Prevention (CDC), accessed 17 July 2017

For incubation at 15 to 20°C, 0.4 to 2.5 Log_{10} CFU/ml of growth of *S. aureus* was observed after 24 hours (Carminati *et al.*, 2008; Donnelly *et al.*, 1968; Janstova *et al.*, 2014). The average growth rate, for a 2.5 Log_{10} CFU/ml increase over 24 hours is equivalent to 0.1 Log_{10} CFU/ml hour. Which is consistent with the maximum growth rates given in Figure 11.

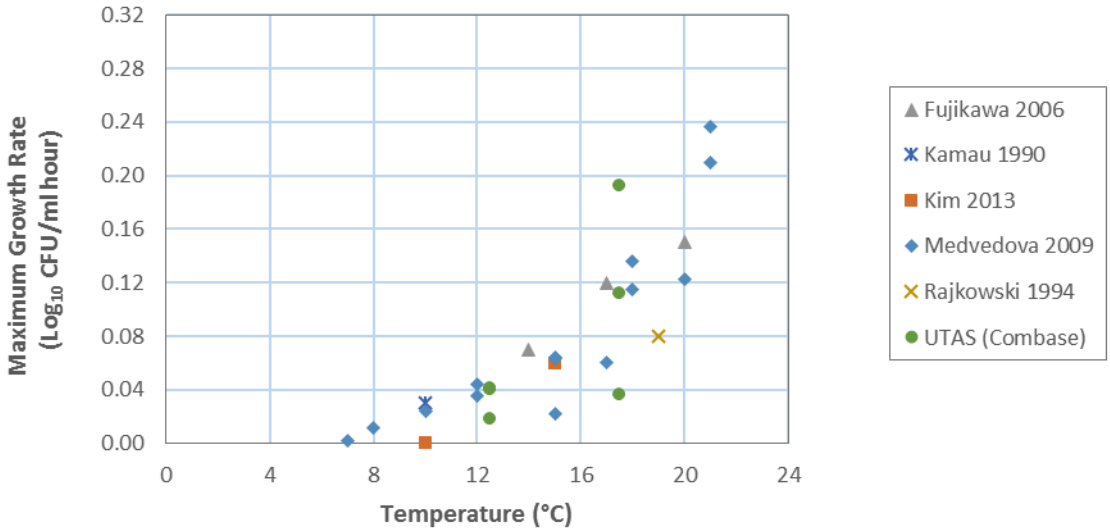


Figure 11: *S. aureus* growth rates in heat treated milk from logarithmic growth phases at different constant temperatures from 7 to 21°C.

11.1.2 Naturally contaminated milk

No growth of *S. aureus* was observed in raw donkey milk incubated at 4 or 20°C for 24 hours (Zhang *et al.*, 2008). The *S. aureus* cell density in the natural microflora of the milk at the start of the experiment was approximately 1.5 Log_{10} CFU/ml.

11.2 GROWTH MODELS

Fujikawa and Morozumi (2006)

Fujikawa et al. (2006) fitted the growth of a single strain of *S. aureus* in sterile milk using a logistic and Barayani and Roberts model (Appendix C.2). Both models fitted the growth data well (Bias 0.989 and 1.03, Accuracy 1.01 and 1.03 [Ross, 1996]). The maximum growth rate was found to be related to temperature via the square root function;

$$\sqrt{k} = 0.0442\,T - 0.239$$

The predicted maximum growth rate by temperature compared to experimental data is shown in Figure 12. The model was generated from experimental data at temperatures greater or equal to 14°C, the model predictions for temperatures above 14°C are plotted as a solid line. For temperatures below 14°C the model predictions are plotted as a dashed line.

The experiments also involved the detection of enterotoxin A. The time toxin was first detected when the cell concentration was approximately $10^{6.5}$. The toxin concentration (ng/ml) was observed to increase linearly with time. The rate constant, p (h^{-1}), for toxin concentration was found to be linearly dependent on temperature, for temperatures between 14 and 32°C.

$$p = 0.0376\,T - 0.559$$

This equation predicts no toxin production for temperatures at 15°C or below.

Kim et al. (2013)

A predictive model based on the Gompertz function (Appendix C.2) was fitted to *S. aureus* (strain KCCM12193) growth curves from experiments conducted at 15, 25 and 35°C in an undefined retail milk (Kim *et al.*, 2013). No growth was detected at 5°C after 48 hours. The maximum growth rate was found to be related to temperature via the function;

$$\sqrt{\mu_{max}} = 0.0231(T - 2.554113)$$

The predicted maximum growth rate by temperature compared to experimental data is shown in Figure 12. The solid line represents the growth predictions in the range of the three temperatures the model was based on (≥ 15°C). The dashed line represents an extrapolation of the model to lower temperatures.

Medvedová et al. (2009)

Three strains of S. aureus (2064, D1, and B1) were separately incubated in UHT milk at temperatures of 7°C to 51°C. In the temperature range of interest, experiments were conducted at 7, 8, 12,15, 18 and 21°C. The growth over time was fitted to the model of Baranyi and Roberts (Appendix C.2). The resulting maximum growth rates were fitted to a function of temperature, for the temperature range 7 to 39°C;

$$\sqrt{k_{2064}} = 0.0420\,T - 0.2057$$
$$\sqrt{k_{D1}} = 0.0455\,T - 0.2381$$
$$\sqrt{k_{B1}} = 0.0390\,T - 0.2739$$

S. aureus strain D1 grew slightly faster than the other two strains (growth rate is plotted against known experimental data in Figure 12).

These models are based on single strains of *S. aureus*, but are based on experiments conducted at multiple incubation temperatures.

11.3 COMPARISON OF GROWTH RATES TO DATA

Figure 12 shows a comparison of the maximum growth rate data presented in Figure 11 to the growth models described in the previous section of this report. All models follow the general trend of the limited milk maximum growth rate data.

Of the three models, the Medvedová model predicts the greatest growth rates at temperatures over 12°C. The model predictions are at the higher range of the experimental data that are plotted in Figure 12, with the exception of one UTAS data point at 17.5°C.

To confirm the usefulness of this model, the model would need to be tested against a greater number of *S. aureus* strains in raw milk.

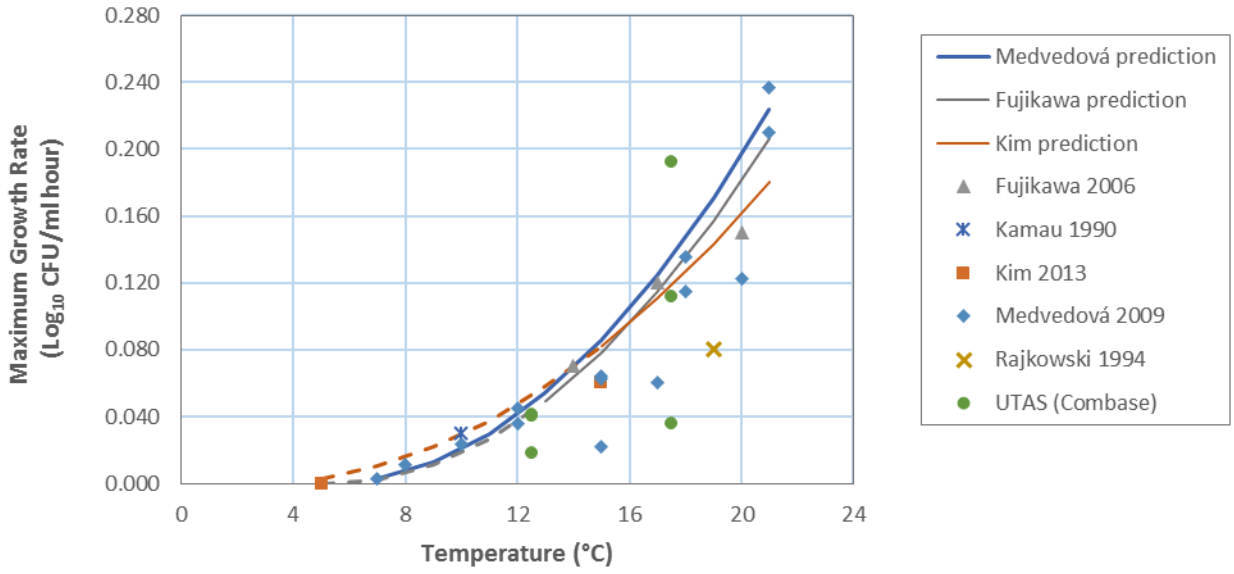


Figure 12: Maximum growth rate experimental data (heat treated milk) and model predictions for *S. aureus* in milk. Dashed lines represent temperatures at which the growth model is extrapolated beyond the experimental data the model is based on.

11.4 CONCLUSIONS

The limited data available to describe the growth of *S. aureus* in milk suggests there will be little growth at temperatures below 10°C, with expected growth below 1 Log_{10} CFU/ml in 24 hours. Above 10°C, the rate of growth increases with temperature, with 1 to 2.5 Log_{10} CFU/ml increase observed over 24 hours at 20°C in raw milk or heat-treated milk. Growth rates at temperatures above 12°C were variable between studies and strains.

The growth of *S. aureus* after 16-24 hours were similar for *S. aureus* inoculated into raw or heat treated milk. One study with un-inoculated donkey milk with an initial *S. aureus* concentration of $10^{1.5}$ CFU/ml did not result in any growth, when incubated at 20°C for 24 hours. It was not clear if the lack of growth was due to strain type, the low initial concentration or other factors. More data would be required to establish if raw milk was associated with lower growth rates, than growth experiments with heat treated milk.

There are currently no growth models based on raw milk data. The Medvedová model for maximum specific growth rate as a function of temperature fits the upper limit of the limited data set well, but further data, including raw milk data would be needed to validate the model for risk assessment purposes.

12. CONCLUSIONS: GROWTH RATES FOR APPLICATION TO CHILLER FAILURE

12.1 RISK ASSESSMENT

To help determine the acceptable amount of microorganism specific growth that can occur during a chiller failure, such that the milk is still fit for purpose as a pasteurised milk product, three components must be defined:

- The expected initial concentration of microorganism s in raw milk in farm vat.
- The growth rate of the microorganism s in raw milk at temperatures representative of chiller failure.
- The acceptable concentration of microorganism s in the raw milk just prior to pasteurisation given both food safety and product quality requirements.

The amount of acceptable microorganism growth is determined by the first and third components. For some microorganism s, factors other than the cell concentration alone must be taken into account. *S. aureus* cells can produce toxins and *Pseudomonas* spp. cells can produce enzymes, both of which are not eliminated during pasteurisation. These by-products can be produced when bacterial concentrations reach 5 Log_{10} CFU/ml (FDA, 2012) and 6-7 Log_{10} CFU/ml (Frank, 2007) respectively. *B. cereus* cells can produce spores which can also survive pasteurisation.

This project has concentrated on the second of these components, the potential growth rates of microorganism s at temperatures in the range 4-20°C in raw milk.

12.2 DATA QUALITY

The literature studies described in this report had sufficient experimental and analysis information to provide confidence in the results being reported for the specific milk, experimental design and the particular microorganism .

The selected literature studies that determined the parameters of the primary growth models (lag time, maximum growth rate and maximum cell density) have all been conducted in heat-treated milk with the exception of one *L. monocytogenes* study and potentially two *B. cereus* studies for which it was unclear which milk type was used.

Most of the studies using raw milk are focused on longer storage times, and typically the first sampling is 16 to 24 hours after incubation. This means it is not possible to determine the growth/inactivation pattern that may be occurring between sampling time points.

Where comparison of the growth rate of microorganisms in raw and heat treated milk is possible, the growth rate in raw milk is at the lower bound of growth rates observed in heat treated milk, with the exception of *S. aureus*.

For each microorganism, there are limited data on maximum growth rates of different strains in the temperature range of 4 to 20°C. The most data available relates to *S. aureus* and *L. monocytogenes*. However, even for these microorganism s more data, especially relating to growth in raw milk, may be required to provide suitable evidence for microorganism specific risk assessment.

12.3 GROWTH RATES

The published maximum growth rates (Log_{10} CFU/ml hour) for the different microorganisms are given in Figure 13. There is high variability in growth estimates both within and between the different microorganisms. At the lower temperatures of 4 to 10°C the highest growth rates were recorded for *Pseudomonas* spp., *B. cereus* and *L. monocytogenes*. While at the higher temperatures close to 20°C, LAB and *B. cereus* have the highest recorded growth rates.

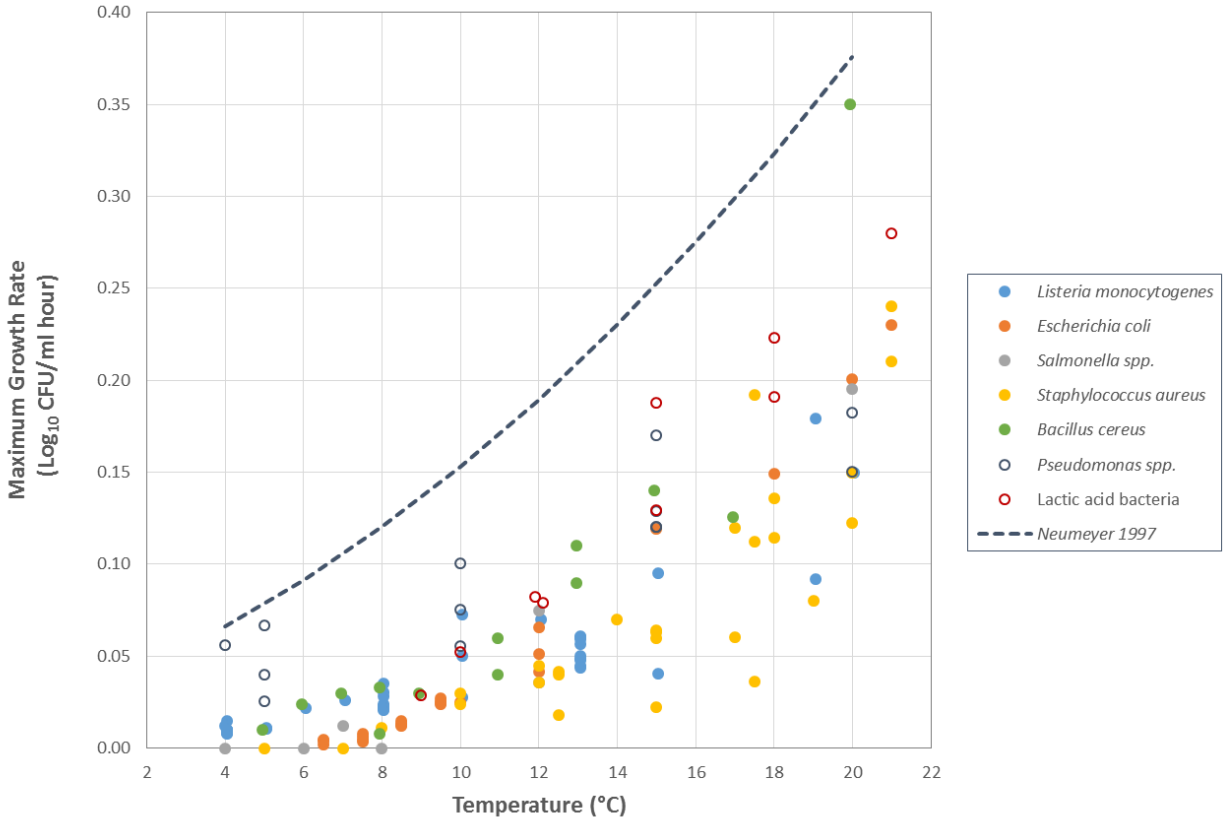


Figure 13: Maximum growth rates from literature studies for different microorganism s and static growth temperatures compared to the *Pseudomonas* model by Neumeyer et al. (1997) given by the dashed line.

Of the observational data (Appendix B) that were collected after given time periods, there is limited growth in the lower temperature range 4-10°C. For example at 10°C, the data suggest most microorganisms would increase in cell concentration by less than 1 Log_{10} CFU/ml in 24 hours. The exceptions include *Pseudomonas* spp. and *L. monocytogenes* which may increase in concentration by 1 Log_{10} CFU/ml after 10-16 hours and 8-13 hours respectively.

At the upper limit of the project temperature range of 20°C, the fastest growing microorganisms were LAB, *B. cereus* and *S. aureus*, with a possible 1 Log_{10} CFU/ml increase in cell concentration after 3-4 hours. These are followed by *Pseudomonas* spp., *E. coli, Salmonella* spp. and *L. monocytogenes* with the potential to produce a 1 Log_{10} CFU/ml increase in cell concentration after 5-6 hours.

The slowest growing microorganisms in the temperature range of 10 to 20°C were Clostridia taking 24 hours at 20°C to grow by 1 Log_{10} CFU/ml in milk. There is no evidence that *Campylobacter* spp. will grow at temperatures below 20°C.

The above summary statements are based on the limited data summarised in this report, and the conclusions may change following future collection of data.

12.4 MODELS FROM THE LITERATURE

Constant temperature growth curves of microorganism s in milk in the published experimental studies followed traditional population growth dynamics, and could be described well by the Gompertz or Baranyi and Roberts models for the specific strains and milk types used.

A square root relationship between the maximum growth rate and temperature has been observed in the studies considered in this report for the temperatures range 4 to 20°C.

Raw milk is not a chemically or microbiologically consistent substrate, with potentially different microflora in different samples which may inhibit or promote the growth of pathogens or spoilage organisms. All but two of the described growth models are specific to the laboratory experiments used to calibrate them. The limited data available for each microorganism makes it impossible to properly evaluate any of the proposed growth models for the purpose of evaluating chiller failure, given the expected variability between study, strain growth rates and the natural microflora of the raw milk.

The models that were designed to recognise study, strain or raw milk variability used two different approaches. Neumeyer et al. (1997) fitted a model to the observed growth rate of a fast growing *Pseudomonas* strain in a broth culture, with the aim of providing an estimate of the greatest likely growth. Augustin et al. (2005) recognised the variability in growth rates of *L. monocytogenes* observed between studies and strains, and fitted bounds on the expected growth rates, based on the data from 14 different studies of liquid dairy products.

Figure 13 shows the Neumeyer model *Pseudomonas* maximum growth rate estimates exceed the observed growth rates of all of the other microorganism s considered. Based on the current data, the model could be used to provide an upper bound for potential microorganism growth during chiller failure. However, the current data only relates to a limited number of data points and strains for each microorganism and further data would be required to provide more confidence in these predictions, given the initial model was based on the growth of a single strain of *Pseudomonas*.

The model by Augustin et al. (2005) provides a potential upper bound estimate of the observed *L. monocytogenes* growth rates, Figure 9. As for the previous model, the maximum growth rates of more strains of *L. monocytogenes* in milk would be needed to provide confidence in the upper bound of the growth model.

12.5 MODELLING APPROACH FOR INITIAL GUIDANCE

The above paragraphs have highlighted there are insufficient data to produce or validate models for estimating the maximum growth of specific microorganisms at given temperatures in raw milk.

The growth rates estimated from studies observing microorganisms in raw milk and heat treated milk suggest that microorganisms will grow faster or at the same rate in heat treated milk, compared with, in raw milk. Therefore, the use of data from heat treated milk studies should be a conservative approach.

To provide some initial guidance on the potential generic microorganism growth during chiller failure, an investigative approach based on the data that have been able to be collected is presented below:

1. A straight line boundary for the maximum growth estimate for a given temperature is chosen. The example given in Figure 14 uses a minimum 0.05 Log_{10} CFU/ml hour safety margin from the observed data points. A straight line relationship between temperature and growth rate has been chosen as part of an investigative tool, rather than trying to model the maximum growth rates as a function of temperature, in which case a square root model may have been more appropriate.
2. The assumption is made that milk is at the maximum temperature reached during the chiller failure for the whole period of chiller failure. It is also assumed there is no lag phase before growth due to change in temperatures. Both assumptions bias the estimates to over-predict the potential growth.
3. Tabulation of the growth scenarios for different growth rate safety margins and temperature/time combinations, to allow potentially safe time/temperature combinations to be determined.

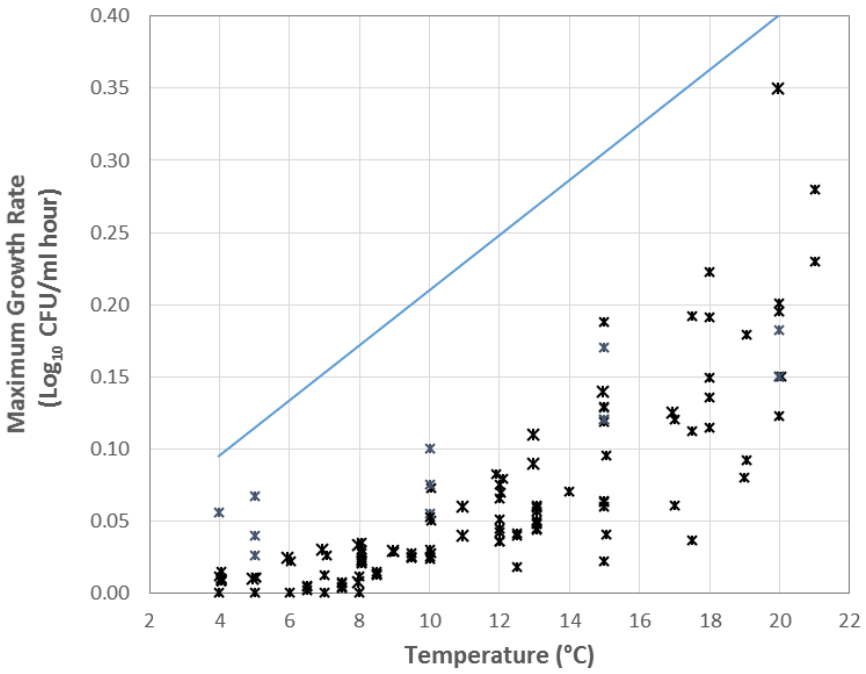


Figure 14: Example of a straight line maximum growth boundary applied to the growth rate data, with a safety margin of 0.05 Log_{10} CFU/ml hour

Table 5 gives the scenario results based on growth rate safety margins of 0.05, 0.1 and 0.2 Log_{10} CFU/ml hour. Based on currently available data, these tables provide a way to determine time/temperature combinations which result in less than a specified amount of growth. For example, with a growth rate safety margin of 0.1 Log_{10} CFU/ml hour, microorganism concentrations would be expected to increase by less than 1 Log_{10} CFU/ml in 2 hours for temperatures of 20°C and below.

For specific time/temperature combinations not shown in the tables, the equations given in Table 5 can be used to estimate microorganism growth.

Table 5: Maximum growth, G, (Log_{10} CFU/ml) scenarios for different growth rate safety margins (GRSM) and time/temperature combinations

(a) GRSM: 0.05 Log_{10} CFU/ml hour G = Hours x (0.027 + 0.0187 x Temperature)

	Hours						
Temperature (°C)	**1**	**2**	**4**	**8**	**12**	**18**	**24**
8	0.2	0.4	0.7	1.4	2.1	3.2	> 5
10	0.2	0.4	0.9	1.7	2.6	3.9	> 5
12	0.3	0.5	1.0	2.0	3.0	4.5	> 5
14	0.3	0.6	1.2	2.3	3.5	> 5	> 5
16	0.3	0.7	1.3	2.6	3.9	> 5	> 5
18	0.4	0.7	1.5	2.9	4.4	> 5	> 5
20	0.4	0.8	1.6	3.2	4.8	> 5	> 5

G ≤ 1
1 < G ≤ 2
2 < G ≤ 3
3 < G ≤ 5
G > 5

(b) GRSM: 0.1 Log_{10} CFU/ml hour G = Hours x (0.077 + 0.0187 x Temperature)

	Hours						
Temperature (°C)	**1**	**2**	**4**	**8**	**12**	**18**	**24**
8	0.2	0.5	0.9	1.8	2.7	4.1	> 5
10	0.3	0.5	1.1	2.1	3.2	4.8	> 5
12	0.3	0.6	1.2	2.4	3.6	> 5	> 5
14	0.3	0.7	1.4	2.7	4.1	> 5	> 5
16	0.4	0.8	1.5	3.0	4.5	> 5	> 5
18	0.4	0.8	1.7	3.3	5.0	> 5	> 5
20	0.5	0.9	1.8	3.6	> 5	> 5	> 5

(c) GRSM: 0.2 Log_{10} CFU/ml hour G = Hours x (0.177 + 0.0187 x Temperature)

	Hours						
Temperature (°C)	**1**	**2**	**4**	**8**	**12**	**18**	**24**
8	0.3	0.7	1.3	2.6	3.9	> 5	> 5
10	0.4	0.7	1.5	2.9	4.4	> 5	> 5
12	0.4	0.8	1.6	3.2	4.8	> 5	> 5
14	0.4	0.9	1.8	3.5	> 5	> 5	> 5
16	0.5	1.0	1.9	3.8	> 5	> 5	> 5
18	0.5	1.0	2.1	4.1	> 5	> 5	> 5
20	0.6	1.1	2.2	4.4	> 5	> 5	> 5

APPENDIX A: METHODOLOGY

A.1 Systematic Literature Review

To capture as many relevant citations as possible, three scientific databases (Pub Med, Web of Science and Science Direct) were searched to identify primary studies of the effects of temperature on the growth of microorganisms in raw milk. The three databases provided a wide coverage of the topic and despite some overlap in results, each database provided enough unique material to warrant inclusion. The search strategy was the same for all three databases with the same key words used for the search, results of which are summarised in Table 6 below. The search string was chosen after initial searches with less defined keywords revealed a huge number of search results that included a high percentage of irrelevant publications.

Table 6. Summary of search strategies and number of results

Database	Keywords	Number of search results
Pub Med	((milk) AND (**raw OR unprocessed OR unpasteur***) AND (growth* OR growth model OR growth/no growth OR activity) AND (micro* OR bacterio* OR protease OR lipase OR psychrotroph* OR Listeria OR L. monocytogenes OR Sal* OR Campy* OR STEC OR VTEC OR shiga* OR enterotoxin OR Escherich* OR E. coli OR Bacillus OR B. cereus OR staphly* OR S. aureus OR Pseudomon* OR Clost* OR Lacto* OR lactic*))	719
Science Direct	As above, filtered by topic 'milk'	1256
Web of science	As above	1926
Pub Med	milk AND **pasteur*** AND (growth* OR growth model OR growth/no growth OR activity) AND (micro* OR bacterio* OR protease OR lipase OR psychrotroph* OR Listeria OR L. monocytogenes OR Sal* OR Campy* OR STEC OR VTEC OR shiga* OR enterotoxin OR Escherich* OR E. coli OR Bacillus OR B. cereus OR staphly* OR S. aureus OR Pseudomon* OR Clost* OR Lacto* OR lactic*)	493
Science Direct	As above, filtered by topic 'milk'	1263
Web of science	As above	1132

All search results were imported into a bibliographic database (EndNote X8) and all duplicates removed. One search was done addressing raw milk and a second search aimed at pasteurised milk. After removing duplicates the latter provided 20 additional articles that were added to the database. The literature search resulted in 3315 citations from which potential relevant studies were selected for the review based on their title and abstract. Of these the full papers of 302 citations were assessed for relevance to the research questions, as outlined in the introduction and in Appendix A.3.

A.2 Combase Data

The Combase database (www.combase.cc, accessed July 2017) was searched for each bacterium at a time. The matrix was set to “milk” and the temperature range to 4 to 22°C. Unpublished data were extracted for *Salmonella* spp. (2), *Listeria monocytogenes* (4), *Staphylococcus aureus* (6), *Lactic acid bacteria* (2) and *Bacillus cereus* (11).

The Combase software was used to fit a growth curve to experimental data, in order to extract the maximum growth rate values. When a lag, log and stationary phase were present in the $\log_{10}$ CFU v time data, a tri-linear relationship was fitted. For data with no lag phase, a bi-linear relationship was used.

A.3 Inclusion and Exclusion Criteria for Data

General inclusion criteria:

- Incubation temperatures in the range 4 to 20°C.
- First enumeration of bacterial growth within 24 hours following inoculation, unless a lag phase is evident, and the growth phase is captured at time intervals of no less than 24 hours.
- Plain milk – which is raw or previously heat treated to reduce local flora. Milk that is whole, or fully or partially skimmed.

Inclusion criteria for growth rates**:**

- Papers/data must have evidence of sufficient samples to be able to identify and calculate a log phase growth rate. Otherwise log increase over a given time period will be reported.
- Papers must provide either a plot of the growth curves, or detailed description of the method used to estimate the maximum growth rate which is sufficient to provide some confidence around the growth rate estimate.

Exclusion criteria:

- Milk with any added ingredients, such as chemicals, plant extracts, inhibitory substances, or ingredients used to change the pH or water activity of the milk.
- Bacterial growth in human milk and non-animal milks, such as soy milk, coconut milk.
- Dairy products other than milk, such as cheese, yoghurt and other fermented milk products, ice cream, flavoured milks.

APPENDIX B: SUPPLEMENTARY GROWTH DATA

Table 7: Observed *B. cereus* growth in milk at different constant temperatures

Temperature (°C)	Milk Treatment	*B. cereus* Growth (Log_{10} CFU/ml)		Milk Type	*E.coli* Strains	Reference (First Author, Year)
		Time (h)	Increase in log count			
8	Pasteurised	24	EP: ~0.7		CCM 2010	Necidová 2014
8	Pasteurised	24	EP: <0.5	Goat milk	CCM 2010	Necidová 2014
8	Pasteurised	24	EP: No Growth	Sheep milk	CCM 2010	Necidová 2014
8 and 15	Raw	24	EP: Inactivation		CCM 2010	Necidová 2014
15	Pasteurised	24	EP: ~2.0		CCM 2010	Necidová 2014
15	Pasteurised	24	EP: ~2.0	Goat milk	CCM 2010	Necidová 2014
15	Pasteurised	24	EP: ~2.0	Sheep milk	CCM 2010	Necidová 2014
16	Pasteurised, ph 6.2	16	EP: ~1		Mixed cocktail of 10 strains	FSA, UK Combase [M282_39]
16	Pasteurised, ph 6.2	22	EP: ~1.7		Mixed cocktail of 10 strains	FSA, UK Combase [M282_39]
20	Reconstituted skim milk	12	EP: 0.2 - 2		Naturally occurring – no additional inoculum	Rodriquez 1986
20	Reconstituted skim milk	24	EP: 3.5 - 5		Naturally occurring – no additional inoculum	Rodriquez 1986

EP: End point growth – difference between CFU concentration at time zero and sampling time point, an inability to establish the extent of any lag phase in growth that may have been present. For *B. cereus*, the increase in CFU count may be due to a mixture of spore germination and cell growth.

Table 8: Observed *E. coli* growth in milk at different constant temperatures

Temperature (°C)	Milk Treatment	*E. coli* Growth (Log_{10} CFU/ml)		Milk Type	*E. coli* Strains	Reference (First Author, Year)
		Time (h)	Increase in log count			
Cooling target temperature range 4 to 6°C						
4	Raw	24	EP: Inactivation	Whole milk	O157:H7	Alhelfi 2012
4	Raw	2-24	NLP: Inactivation	Whole milk	O157:H7 933	Farrag 1992
4	Raw	24	EP: Inactivation	Whole milk	O157:H7 ATCC 43895	Zarei 2010
5	Raw	24	EP: No growth	Whole milk	5 strains of O157:H7	Wang 1997
4	Pasteurised	24	EP: Inactivation	Whole milk	O157:H7	Alhelfi 2012
4	UHT	24	EP: <0.3 then inactivation	Whole milk	O157:H7 [CECT 4267 and 4067]	Mamani 2003
Raw milk with incubation temperature above the target of 6°C						
5-9	Raw	24	EP: No growth	Whole milk	ATCC 25922, O127:H6, O5:H-	McLean 2013
8	Raw (left to separate)	16	EP: No growth	Whole milk	Cocktail of O157:H7 [ATCC 43894, ATCC 700599, DSM 8579, IZSLER 643]	Carminati 2008
8	Raw	24	EP: No growth	Whole milk	5x O157:H7	Wang 1997
8	Raw	24	EP: 0.6	Goats milk	3 goat milk strains	Zapico 1995
15	Raw (left to separate)	16	EP: No growth	Whole milk	O157:H7 [ATCC 43894, ATCC 700599, DSM 8579, IZSLER 643]	Carminati 2008
15	Raw	24	EP: ~1.5	Whole milk	5x O157:H7	Wang 1997
20	Raw	24	EP: ~1.3	Whole milk	O157:H7	Alhelfi 2012
20	Raw (left to separate)	16	EP: ~1	Skim milk component	O157:H7 [ATCC 43894, ATCC 700599, DSM 8579, IZSLER 643]	Carminati 2008
20	Raw (left to separate)	16	EP: ~3.5	Separated cream component	O157:H7 [ATCC 43894, ATCC 700599, DSM 8579, IZSLER 643]	Carminati 2008
20	Raw	24	EP: ~3	Whole milk	O157:H7 [ATCC 43895]	Zarei 2010

Temperature (°C)	Milk Treatment	*E.coli* Growth (Log_{10} CFU/ml)		Milk Type	*E.coli* Strains	Reference (First Author, Year)
		Time (h)	Increase in log count			
Heat treated milk with incubation temperature above the target of 6°C						
5-9	UHT	24	EP: No growth	Whole milk	ATCC 25922, O127:H6, O5:H-	McLean 2013
8	UHT	24	EP: No growth	Whole milk	5x O157:H7	Wang 1997
15	UHT	24	EP: ~2	Whole milk	5x O157:H7	Wang 1997
20	Pasteurised	24	EP: 1.8	Whole milk	O157:H7	Alhelfi 2012
20	UHT	24	EP: 1	Whole milk	O157:H7 [CECT 4267, 4067]	Mamani 2003

NLP: No lag phase evident in plot.

EP: End point growth – difference between CFU concentration at time zero and sampling time point, an inability to establish the extent of any lag phase in growth that may have been present.

Table 9: Observed *L. monocytogenes* growth in milk at different constant temperatures

Temperature (°C)	Milk Treatment	*L. monocytogenes* Growth (Log_{10} CFU/ml)		Milk Type	Listeria Strains	Reference (First Author, Year)
		Time (h)	Increase in log count			
Temperature range 4 to 6°C						
4	Raw	24	EP: 0.1	Whole milk	Ohio	Rodriguez 1997
4	Raw	5	No Growth	Whole milk	ATCC 6994, plus 2 wild raw milk strains	Giacometti 2012
4	Raw	8	No Growth	Whole Milk	Scott A, 5069, ATCC 19119, NCTC 1194	Gaya 1991
4	Raw	24	No Growth	Whole Milk	Scott A, 5069, ATCC 19119, NCTC 1194	Gaya 1991
4	Raw	24	No Growth	Goats Milk	Scott A, 5069, NCTC 11994	Zapico 1993
4	Pasteurised	24	ALP: 0.2 to 1	Whole and skim milk	F5027, F5069, 19111,19113,19115	Donnelly 1986
4	UHT	24	ALP: <0.2	Half skim milk	ATCC 19111, serotype 1/2a	Denis 1989
Raw milk with incubation temperature above the target of 6°C						
8	Raw	24	EP: 0.2	Whole milk	Ohio	Rodriguez 1997
8	Raw	8	No Growth	Whole Milk	Scott A, 5069, ATCC 19119, NCTC 1194	Gaya 1991
8	Raw	24	No Growth	Whole Milk	Scott A, 5069, ATCC 19119, NCTC 1194	Gaya 1991
8	Raw	24	No Growth	Goats Milk	Scott A, 5069, NCTC 11994	Zapico 1993
8	Raw (left to separate)	16	No Growth	Whole milk	Cocktail of ATCC 19114, Scott A, ILC 23, ILC 34	Carminati 2008
10	Raw	24	EP: 0.5	Whole and skim milk	Scott A	Marshall 1988
15	Raw (left to separate)	16	No Growth	Whole milk	Cocktail of ATCC 19114, Scott A, ILC 23, ILC 34	Carminati 2008

Temperature (°C)	Milk Treatment	*L. monocytogenes* Growth (Log_{10} CFU/ml)		Milk Type	Listeria Strains	Reference (First Author, Year)
		Time (h)	Increase in log count			
Raw milk with incubation temperature above the target of 6°C (continued)						
20	Raw	8	No Growth	Goats Milk	Scott A, 5069	Zapico 1993
20	Raw	8	EP: 0.8	Goats Milk	NCTC 11994	Zapico 1993
20	Raw	24	EP: 0.6-1.5	Goats Milk	Scott A, 5069	Zapico 1993
20	Raw	24	EP: 2.3	Goats Milk	NCTC 11994	Zapico 1993
20	Raw (left to separate)	16	EP: ~1	Skim milk	Cocktail of ATCC 19114, Scott A, ILC 23, ILC 34	Carminati 2008
20	Raw (left to separate)	16	EP: ~3	Separated cream	Cocktail of ATCC 19114, Scott A, ILC 23, ILC 34	Carminati 2008
Heat treated milk with incubation temperature above the target of 6°C						
10	Heated 20 minutes at 57°C	24	EP: 0.8	Whole milk	Scott A	Kamau 1990
10	Pasteurised	24	ALP: 1.5 to 3	Whole and skim milk	F5027, F5069, 19111,19113,19115	Donnelly 1986
15	UHT	24	NLP: ~1.5	Half skim milk	ATCC 19111, serotype 1/2a	Denis 1989

ALP: Growth over time period, time starting after the initial lag phase,

NLP: No lag phase evident in plot.

EP: End point growth – difference between CFU concentration at time zero and sampling time point, unable to establish the extent of any lag phase in growth that may have been present.

Table 10: Observed *S. aureus* growth in milk at different constant temperatures

Temperature (°C)	Milk Treatment	*S. aureus* Growth (Log_{10} CFU/ml)		Milk Type	*S. aureus* Strains	Reference (First Author, Year)
		Time (h)	Increase in log count			
Temperature range 4 to 6°C						
4	Raw (previously frozen)	48	EP: No Growth	Donkey milk	In milk (1.5 log/ml initial count)	Zhang 2008
Raw milk with incubation temperature above the target of 6°C						
8	Raw (left to separate)	16	EP: No growth	Whole milk	Cocktail of ATCC 19095, ATCC 25923, ATCC 14458, ILC 1A	Carminati 2008
10	Raw	24	EP: No Growth		MF 224 type A	Donnelly 1968
15	Raw	24	EP: ~0.4	Cow Whole milk	CCM 5971	Janštová 2014
15	Raw (left to separate)	16	EP: No growth	Whole milk	Cocktail of ATCC 19095, ATCC 25923, ATCC 14458, ILC 1A	Carminati 2008
20	Raw	24	EP: 2-2.5	Standard plate count 10^4	MF 224 type A	Donnelly 1968
20	Raw	24	EP: <1.0	Standard plate count 10^6	MF 224 type A	Donnelly 1968
20	Raw (left to separate)	16	EP: ~1	Skim milk component	Cocktail of ATCC 19095, ATCC 25923, ATCC 14458, ILC 1A	Carminati 2008
20	Raw (left to separate)	16	EP: ~3	Separated cream component	Cocktail of ATCC 19095, ATCC 25923, ATCC 14458, ILC 1A	Carminati 2008
20	Raw (previously frozen)	24	EP: No Growth	Donkey milk	Already in milk (1.5 log CFU/ml initial count)	Zhang 2008
Heat treated milk with incubation temperature above the target of 6°C						
10	UHT	24	EP: No Growth		Fourteen different strains	Valihrach 2013
10	Pasteurised	24	EP: No Growth		MF 224 type A	Donnelly 1968
15	Pasteurised	24	EP: ~1.7	Cow whole milk	CCM 5971	Janštová 2014
20	Pasteurised	24	EP: 1.5-2.5		MF 224 type A	Donnelly 1968

EP: End point growth – difference between CFU concentration at time zero and sampling time point, unable to establish the extent of any lag phase in growth that may have been present.

APPENDIX C: GROWTH MODELS

C.1 Growth Rates

Most bacterial microorganism growth at a constant incubation temperature follows a sigmoidal form of growth curve as shown in Figure 15.

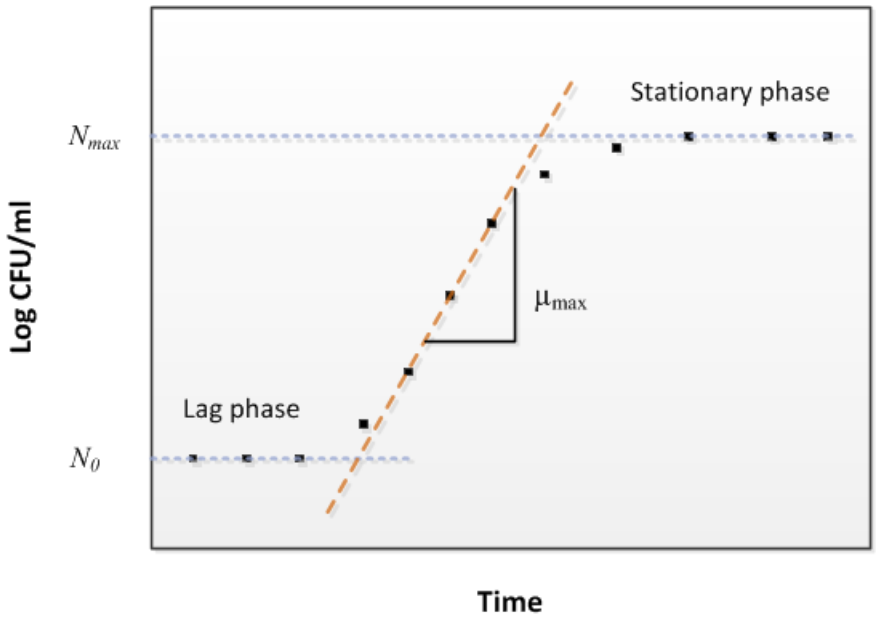


Figure 15: Sigmoidal Growth Curve

Where;

$N(t)$ is the cell concentration at time t

N_o is the initial level at t=0

N_{max} is the maximal cell density

A good introduction to the growth models is provided by Devlieghere et al. (2009). The literature in this report refers to the following forms of models.

C.2 Primary Models

Primary growth models describe how a population of culturable microorganisms change in number over time.

Gompertz Function

The Gompertz function expresses the Log concentration of microorganisms over time using the sigmoidal shape shown in Figure 15.

$$\log N(t) = N_0 + De^{-e^{-B(t-M)}} \quad \textbf{(1)}$$

The model parameters, B and D relate to the growth curve (Figure 15) with the following relationships:

$N_{max} = N_0 + D, \qquad \mu_{max} = BD/e^1$,

and M is the time at which the growth rate is greatest.

Logistic Model

A logistic form of the Gompertz function has been used to express the Log concentration of microorganisms over time. The logistic model is symmetrical unlike the Gompertz function.

$$\log N(t) = \frac{N_{max} - N_0}{N_0 + e^{(-B(t-M))}} \quad \textbf{(2)}$$

Baranyi and Roberts (1994)

This model uses first order differential equations to describe the changes in microorganism concentrations over time, when temperature and the cell population's ability to grow also changes over time.

$$\frac{dN}{dt} = \mu_{max}\ \alpha(t)\ u(t)\ N(t) \quad \textbf{(3)}$$

where α is an adaptation function which provides a smooth transition from the initial cell concentration to the maximum growth phase and u is an inhibition function providing a transition from the maximum to stationary growth phases:

$\alpha(t) = \frac{q(t)}{1+q(t)}$ and $u(N) = 1 - \left(\frac{N(t)}{N_{max}}\right)^m$,

The function q represents the physiological state of the cells and their associated ability to grow. The constant m defines the curvature of the growth rate moving from maximum to stationary growth.

C.3 Secondary Models

Secondary growth models define how the growth rates changes depending on environmental factors such as temperature or pH.

Square root model

The dependence of a growth rate k on temperature for temperatures lower than the optimum growth temperatures can often be described by a square root relationship:

$$\sqrt{k} = b(T - T_{min}) \quad \textbf{(4)}$$

where b is a constant and T_{min} is the extrapolation of the regression equation to a point of zero growth and does not define the lowest observed temperature at which growth has been observed.

Extended square root model

The square root model can be extended for the whole temperature range at which growth can occur, by including a factor which includes a maximum temperature parameter, T_{max} and a constant c:

$$\sqrt{k} = b(T - T_{min})\left(1 - e^{c(T - T_{max})}\right) \quad \textbf{(5)}$$

Cardinal models

The growth rate is described as a function of temperature, pH and water activity, and their cardinal values; optimum, minimum and maximum for growth;

$$\mu_{max}(T, pH, a_W) = \mu_{opt}\ \tau\left(T, T_{opt}, T_{min}, T_{max}\right) \times \rho\left(pH, pH_{opt}, pH_{min}, pH_{max}\right) \times \alpha\left(a_W, a_{W\,opt}, a_{W\,min}, a_{W\,max}\right)$$

REFERENCES

Acai P, Valik L, Medved'ova A, Rosskopf F. (2016) Modelling and predicting the simultaneous growth of *Escherichia coli* and lactic acid bacteria in milk. *Food Science and Technology International*; 22(6): 475-484.

Alavi SH, Puri VM, Knabel SJ, Mohtar RH, Whiting RC. (1999) Development and validation of a dynamic growth model for *Listeria monocytogenes* in fluid whole milk. *J Food Prot*; 62(2): 170-176.

Alhelfi NA, Lahmer RA, Jones DL, Williams AP. (2012) Survival and metabolic activity of lux-marked *Escherichia coli* O157:H7 in different types of milk. *J Dairy Res*; 79(3): 257-261.

Augustin JC, Zuliani V, Cornu M, Guillier L. (2005) Growth rate and growth probability of *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions. *J Appl Microbiol*; 99(5): 1019-1042.

Bao H, Zhang P, Zhang H, Zhou Y, Zhang L, Wang R. (2015) Bio-Control of *Salmonella* Enteritidis in Foods Using Bacteriophages. *Viruses*; 7(8): 4836-4853.

Baranyi J, Roberts TA. (1994) A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology*. 23(3-4) 277-294.

Barrell RA. (1981) The survival of *Campylobacter coli/jejuni* in unpasteurised milk. *J Infect*; 3(4): 348-352.

Bergdoll MS, Lee Wong AC. (2006) Staphylococcal intoxications. In: HP Reimann; DO Cliver (eds). Foodborne Infections and Intoxications. Elsevier.

Bates JR, Bodnaruk PW. (2003) Clostridium perfringens. In: AD Hocking (eds). Foodborne Microorganisms of Public Health Significance. NSW, Australia: AIFST and Southwood Press.

Bovill R, Bew J, Cook N, D'Agostino M, Wilkinson N, Baranyi J. (2000) Predictions of growth for *Listeria monocytogenes* and *Salmonella* during fluctuating temperature. *Int J Food Microbiol*; 59(3): 157-165.

Bramley AJ, McKinnon CH. (1990) The Microbiology of Raw Milk. In: R R.K. (eds). Dairy Microbiology: The Microbiology of Milk. England: Elsevier Science

Buchanan RL, Klawitter LA. (1991) Effect of temperature history on the growth of *Listeria monocytogenes* Scott A at refrigeration temperatures. *Int J Food Microbiol*; 12(2-3): 235-245.

Carminati D, Bonvini B, Neviani E, Mucchetti G. (2008) The fate of pathogenic bacteria during the spontaneous creaming process of raw milk: a laboratory-scale study. *Milchwissenschaft-Milk Science International*; 63(4): 416-419.

Christopher FM, Smith GC, Vanderzant C. (1982) Effect of Temperature and pH on the Survival of *Campylobacter fetus*. *J Food Prot*; 45(3): 253-259.

Cox JM, MacRae IC. (1988) Growth of psychrotrophic bacteria in raw and UHT-treated goats' milk. *J Appl Bacteriol*; 64(5): 403-407.

De Jonghe V, Coorevits A, Van Hoorde K, Messens W, Van Landschoot A, De Vos P, Heyndrickx M. (2011) Influence of storage conditions on the growth of *Pseudomonas* species in refrigerated raw milk. *Appl Environ Microbiol*; 77(2): 460-470.

de Oliveira GB, Favarin L, Luchese RH, McIntosh D. (2015) Psychrotrophic bacteria in milk: How much do we really know? *Brazilian Journal of Microbiology*; 46(2): 313-321.

Denis F, Ramet JP. (1989) Antibacterial activity of the lactoperoxidase system on *Listeria-monocytogenes* in trypticase soy broth, uht milk and french soft cheese. *J Food Prot*; 52(10): 706-711.

Devlieghere F, Francois K, Vermeulen A, Debevere J. (2009) Predictive Microbiology. In: R Costa; K Kristbergsson (eds). Predictive Modeling and Risk Assessment. New York: Springer.

Donnelly C, Briggs E. (1986) Psychrotrophic growth and thermal inactivation of *Listeria monocytogenes* as a function of milk composition. *J Food Prot*; 49(12): 994–998.

Donnelly CB, Leslie JE, Black LA. (1968) Production of enterotoxin A in milk. *Appl Microbiol;* 16(6): 917-924.

Doyle MP, Roman DJ. (1982) Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. *Appl Environ Microbiol*; 44(5): 1154-1158.

Dufeu J, Leesment H. (1974) Growth and resisitance charactersitcis of some psychrotrophic sporeformers isolated from raw milk. In: (eds). Proceedings of XIX International Dairy Congress, India. pp: 535-536.

Farber JM, Sanders GW, Speirs JI. (1990) Growth of *Listeria-monocytogenes* in naturally-contaminated raw-milk. *Lebensmittel-Wissenschaft & Technologie*; 23(3): 252-254.

Farrag SA, Elgazzar FE, Marth EH. (1992) Behavior of Escherichia coli 157-H7 or Yersinia enterocolitica at 4 degrees-C or 7 degrees-C in raw milk inoculated with a commercial culture of lactic-acid bacteria. *Milchwissenschaft-Milk Science International*. 47(3)149-152.

Food and Drug Administration (FDA). (2012) *Staphylococcus aureus* in Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins. Second Edition. https://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf

Food Standards Agency (FSA), UK, data derived from Combase database, www.combase.cc, accessed July 2017

Food Standards Australia New Zealand (FSANZ). (2009) Microbiological Risk Assessment of Raw Cow Milk. http://www.foodstandards.govt.nz/code/proposals/documents/P1007%20PPPS%20for%20raw%20milk%201AR%20SD1%20Cow%20milk%20Risk%20Assessment.pdf

Franciosi E, Settanni L, Cavazza A, Poznanski E. (2009) Biodiversity and technological potential of wild lactic acid bacteria from raw cows' milk. *International Dairy Journal*; 19(1): 3-11.

Frank JF. (2007) Milk and Dairy Products. In: MP Doyle; LR Beuchat (eds). Food Microbiology: Fundamentals and Frontiers. Washington D.C.: ASM Press.

Fujikawa H, Morozumi S. (2006) Modeling *Staphylococcus aureus* growth and enterotoxin production in milk. *Food Microbiology*; 23(3): 260-267.

Gaya P, Medina M, Nunez M. (1991) Effect of the lactoperoxidase system on *Listeria monocytogenes* behavior in raw milk at refrigeration temperatures. *Appl Environ Microbiol;* 57(11): 3355-3360.

Giacometti F, Serraino A, Finazzi G, Daminelli P, Losio MN, Tamba M, Garigliani A, Mattiolo R, Riu R, Zanoni RG. (2012) Field handling conditions of raw milk sold in vending machines : experimental evaluation of the behaviour of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Campylobacter jejuni*. *Italian Journal of Animal Science*; 11(e24): 132:136.

Giacometti F, Bardasi L, Merialdi G, Morbarigazzi M, Federici S, Piva S, Serraino A. (2016) Shelf life of donkey milk subjected to different treatment and storage conditions. *J Dairy Sci*; 99(6): 4291-4299.

Gilmour A, Rowe MT. (1990) Microorganisms associated with milk. In: RK Robinson (eds). Dairy Microbiology Handbook. The microbiology of milk. Elsevier Applied Science.

Granum PE. (2007) Bacillus cereus. In: MP Doyle; LR Beuchat (eds). Food Microbiology: Fundamentals and Frontiers. Washington, D.C.: ASM Press.

Greene VW, Jezeski JJ. (1954) Influence of Temperature on the Development of Several Psychrophilic Bacteria of Dairy Origin. Applied Microbiology; 2(2): 110-117.

Hill B, Smythe B, Lindsay D, Shepherd J. (2012) Microbiology of raw milk in New Zealand. *Int J Food Microbiol*; 157(2): 305-308.

International Commission on Microbiological Specifications for Foods (ICMSF). (1996) Microorganisms in foods 5: Microbiological specifications of food pathogens. Blackie Academic & Professional.

Janštová B, Necidova L, Skockova A, Janstova B. (2014) *Staphylococcal* enterotoxin production in model samples of milk and fresh cheese. *Journal of Food and Nutrition Research*; 53(4): 389-392.

Jenson I, Moir CJ. (2003) *Bacillus cereus* and other *Bacillus* species. In: AD Hocking (eds). Foodborne Microorganisms of Public Health Significance. NSW, Australia: AIFST.

Kamau DN, Doores S, Pruitt KM. (1990) Antibacterial activity of the lactoperoxidase system against *Listeria-monocytogenes* and *Staphylococcus-aureus* in milk. *J Food Prot*; 53(12): 1010-1014.

Kasrazadeh M, Genigeorgis C. (1994) Potential growth and control of *Salmonella* in Hispanic type soft cheese. *Int J Food Microbiol*; 22(2-3): 127-140.

Kasrazadeh M, Genigeorgis C. (1995) Potential growth and control of *Escherichia coli* O157:H7 in soft hispanic type cheese. *Int J Food Microbiol*; 25(3): 289-300.

Kauppi KL, Tatini SR, Harrell F, Feng P. (1996) Influence of substrate and low temperature on growth and survival of verotoxigenic *Escherichia coli*. *Food Microbiology*; 13(5): 397-405.

Kim HW, Lee SA, Yoon Y, Paik HD, Ham JS, Han SH, Seol KH, Jang A, Park BY, Oh MH. (2013) Development of Kinetic Models Describing Kinetic Behavior of *Bacillus cereus* and *Staphylococcus aureus* in Milk. *Korean Journal for Food Science of Animal Resources*; 33(2): 155-161.

Kumari S, Sarkar PK. (2016) *Bacillus cereus* hazard and control in industrial dairy processing environment. *Food Control*; 69: 20-29.

Larsen HD, Jorgensen K. (1999) Growth of *Bacillus cereus* in pasteurized milk products. *Int J Food Microbiol*; 46(2): 173-176.

Lin H, Shavezipur M, Yousef A, Maleky F. (2016) Prediction of growth of *Pseudomonas fluorescens* in milk during storage under fluctuating temperature. *J Dairy Sci*; 99(3): 1822-1830.

Malacarne M, Summera A, Franceschi P, Formaggioni P, Pecorari M, Panari G, Vecchia P, Sandri S, Fossa E, Scotti C, Mariani P. (2013) Effects of storage conditions on physico-chemical characteristics, salt equilibria, processing properties and microbial development of raw milk. *International Dairy Journal;* 29(1): 36-41.

Mamani Y, Quinto EJ, Simal-Gandara J, Mora MT. (2003) Growth and survival of *Escherichia coli* O157 : H7 in different types of milk stored at 4 degrees C or 20 degrees C. *Journal of Food Science*; 68(8): 2558-2563.

Marshall DL, Schmidt RH. (1988) Growth of *Listeria monocytogenes* at 10°C in Milk Preincubated with Selected Pseudomonads. *J Food Prot*; 51(4): 277-282.

Marshall JC, Soboleva TK, Jamieson P, French NP. (2016) Estimating Bacterial Pathogen Levels in New Zealand Bulk Tank Milk. *J Food Prot*; 79(5): 771-780.

McClane BA. (2007) *Clostridium perfringens*. In: MP Doyle; LR Beuchat (eds). Food Microbiology: Fundamentals and Frontiers. Washington, D.C.: ASM Press.

McLean SK, Dunn LA, Palombo EA. (2013) Phage inhibition of Escherichia coli in ultrahigh-temperature-treated and raw milk. *Foodborne pathogens and disease*. 10(11) 956-62.

Medvedová A, Valik L, Sirotná Z, Liptáková D. (2009) Growth Characterisation of *Staphylococcus aureus* in Milk: a Quantitative Approach. *Czech Journal of Food Sciences;* 27(6): 443-453.

Meer RR, Baker J, Bodyfelt FW, Griffiths MW. (1991) Psychrotrophic *bacillus* spp in fluid milk-products - a review. *J Food Prot*; 54(12): 969-979.

Murphy PM, Rea MC, Harrington D. (1996) Development of a predictive model for growth of *Listeria monocytogenes* in a skim milk medium and validation studies in a range of dairy products. *J Appl Bacteriol*; 80(5): 557-564.

Necidova L, Bursova S, Skockova A, Janstova B, Pracharova P, Sevcikova Z, Janstova B. (2014) Growth and enterotoxin production of *Bacillus cereus* in cow, goat, and sheep milk. *Acta Veterinaria Brno*; 83: S3-S8.

Neumeyer K, Ross T, Thomson G, McMeekin TA. (1997) Validation of a model describing the effects of temperature and water activity on the growth of psychrotrophic pseudomonads. *Int J Food Microbiol*; 38(1): 55-63.

Orr KE, Lightfoot NF, Sisson PR, Harkis BA, Tweddle JL, Boyd P, Carroll A, Jackson CJ, Wareing DR, Freeman R. (1995) Direct milk excretion of *Campylobacter jejuni* in a dairy cow causing cases of human enteritis. *Epidemiol Infect*; 114(1): 15-24.

Park SF. (2002) The physiology of *Campylobacter* species and its relevance to their role as foodborne pathogens. *Int J Food Microbiol*; 74(3): 177-188.

Paulin S, Horn B, Hudson JA. (2012) Factors influencing staphylococcal enterotoxin production in Dairy Products. 2012/07. ESR report prepared for the Ministry for Primary Industries.

Pearson LJ, Marth EH. (1990) Behavior of *Listeria-monocytogenes* in the presence of cocoa, carrageenan, and sugar in a milk medium incubated with and without agitation. *J Food Prot*; 53(1): 30-37.

Rajkowski KT, Calderone SM, Jones E. (1994) Effect of polyphosphate and sodium chloride on the growth of *Listeria monocytogenes* and *Staphylococcus aureus* in ultra-high temperature milk. *J Dairy Sci;* 77(6): 1503-1508.

Rasooly R, Do PM. (2010) *Clostridium botulinum* neurotoxin type B is heat-stable in milk and not inactivated by pasteurization. *J Agric Food Chem*; 58(23): 12557-12561.

Read Jr RB, Bradshaw JG, Francis DW. (1970) Growth and Toxin Production of *Clostridium botulinum* Type E in Milk. *J Dairy Sci*; 53(9): 1183-1186.

Rodriguez E, Tomillo J, Nunez M, Medina M. (1997) Combined effect of bacteriocin-producing lactic acid bacteria and lactoperoxidase system activation on *Listeria monocytogenes* in refrigerated raw milk. *J Appl Microbiol*; 83(3): 389-395.

Rodriquez MH, Barrett EL. (1986) Changes in microbial-population and growth of *Bacillus-cereus* during storage of reconstituted dry milk. *J Food Prot*; 49(9): 680-686.

Rosenow EM, Marth EH. (1987) Growth of *Listeria monocytogenes* in Skim, Whole and Chocolate Milk, and in Whipping Cream during Incubation at 4, 8, 13, 21 and 35°C. *J Food Prot*; 50(6): 452-459.

Ross T. (1996) Indices for performance evaluation of predictive models in food microbiology. *J Food Microbiol*; 81:501-508.

Salter MA, Ross T, McMeekin TA. (1998) Applicability of a model for non-pathogenic *Escherichia coli* for predicting the growth of pathogenic *Escherichia coli. J Appl Microbiol*; 85(2): 357-364.

Shelley AW, Deeth HC, MacRae IC. (1986) Growth of lipolytic psychrotrophic pseudomonads in raw and ultra-heat-treated milk. *J Appl Bacteriol*; 61(5): 395-400.

Simms L, Rae ICM. (1989) Survival of *Campylobacter-jejuni* in raw, pasteurized and ultra-heat-treated goats milk stored at different temperatures. *Letters in Applied Microbiology*; 8(5): 177-180.

Slovak University of Technology (STU), Slovakia, data derived from Combase database, www.combase.cc, accessed July 2017

University of Tasmania (UTAS), Tasmanian Institute of Agriculture, Australia, data derived from Combase database, www.combase.cc, accessed July 2017

Valbuena E, Barreiro J, Sanchez E, Castro G, Kutchinskaya V, Brinez W. (2008) Growth Prediction of *Lactococcus lactis* subsp *lactis* in Sterile Skimed Milk as a Function of Temperature. *Revista Cientifica-Facultad De Ciencias Veterinarias*; 18(6): 745-758.

Valihrach L, Alibayov B, Demnerova K. (2013) Production of staphylococcal enterotoxin C in milk. *International Dairy Journal*; 30(2): 103-107.

Wang GD, Zhao T, Doyle MP. (1997) Survival and growth of *Escherichia coli* O157:H7 in unpasteurized and pasteurized milk. *J Food Prot*; 60(6): 610-613.

Xanthiakos K, Simos D, Angelidis AS, Nychas GJ, Koutsoumanis K. (2006) Dynamic modeling of *Listeria monocytogenes* growth in pasteurized milk. *J Appl Microbiol*; 100(6): 1289-1298.

Zapico P, Gaya P, Nunez M, Medina M. (1993) Goats milk lactoperoxidase system against *Listeria-monocytogenes*. *J Food Prot*; 56(11): 988-990.

Zapico P, Gaya P, Nunez M, Medina M. (1995) Activity of goats milk lactoperoxidase system on *Pseudomonas-fluorescens* and *Escherichia-coli* at refrigeration temperatures. *J Food Prot*; 58(10): 1136-1138.

Zarei M, Shekarforoush SS, Khajehali E, Nazer AHK. (2010) Antibacterial activity of lactoperoxidase system, activated by two different recommended methods against *Escherichia coli* O157:H7 in raw milk. *Milchwissenschaft-Milk Science International*; 65(3): 298-301.

Zhang XY, Zhao L, Jiang L, Dong ML, Ren FZ. (2008) The antimicrobial activity of donkey milk and its microflora changes during storage. *Food Control;* 19(12): 1191-1195.

Zwietering MH, deWit JC, Notermans S. (1996) Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. *Int J Food Microbiol*; 30(1-2): 55-70.

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