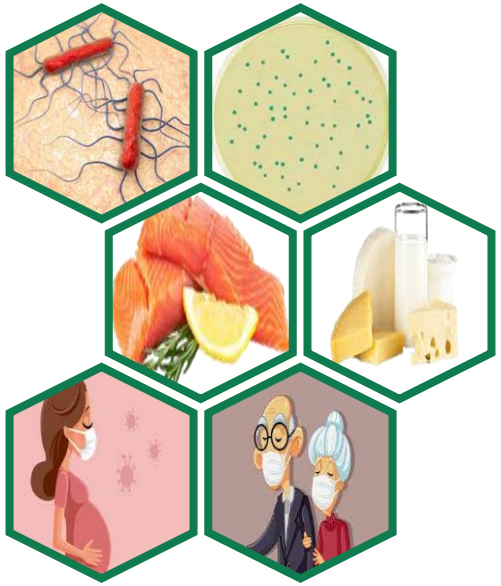
Listeria monocytogenes growth in a semi-soft, rind-ripened cheese at cold chain and abuse temperatures

Peter Myintzaw, PhD
Postdoctoral researcher, Food Biosciences Dept
Teagasc Food Research Centre

The evidence of recent outbreak that involve loss of lives and many hospitalisation caused by *Listeria monocytogenes* indicated that this deadly foodborne pathogen is still a serious concern for public health.



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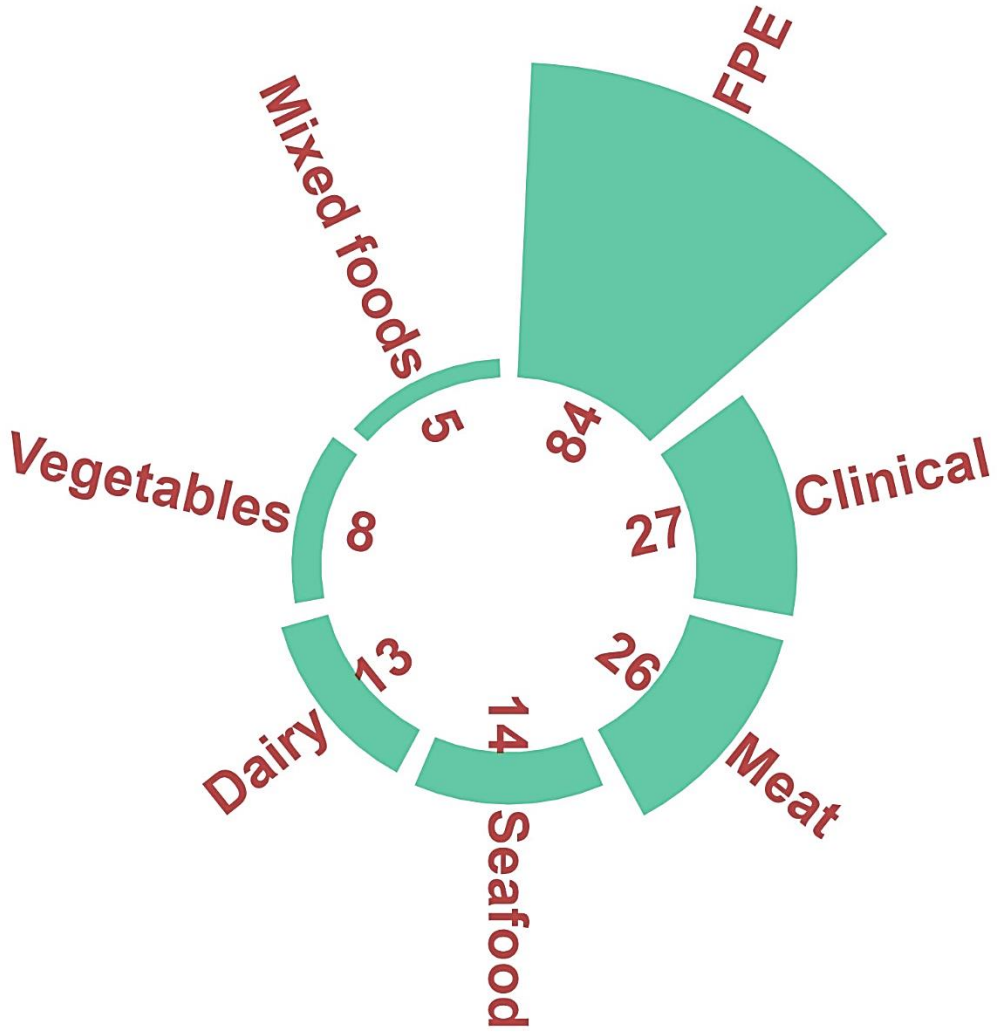
Outline

- Overview of challenge studies project
- *Lm* strains background
- Reason for chosen food matrix
- Cold tolerance screening
- Preliminary study
- Challenge study
- Final thought

Project brief

Project title:	Understanding *Lm* growth in food in order to simplify the guidelines for undertaking food challenge studies. Ref: 17F244
Task 1:	Challenge studies to determine the *Lm* growth in food. (Teagasc, UL, UCD).
Task 2:	Examining genetic traits that determine growth on food. (Teagasc, NUIG, UL).
Task 3:	Utilising WGS, to study the genetic basis of stress tolerance. (UL, UCD).
Task 4:	Virulent vs avirulent strains on ability to growth in food. (UCC, Teagasc).
Task 5:	Predictive modelling of *Lm* growth in food . (UCD, Teagasc, UL).

Teagasc Moorepark *Lm* strains collection (*n* = 177)



Phenotype/Genotype Association Studies at Teagasc

Strain variation in sanitizer tolerance (*n* = 150) (Divosan VT3 & QAC)

Strain variation in cold tolerance (*n* = 150) (4, 10, 15, 20 and 30°C)

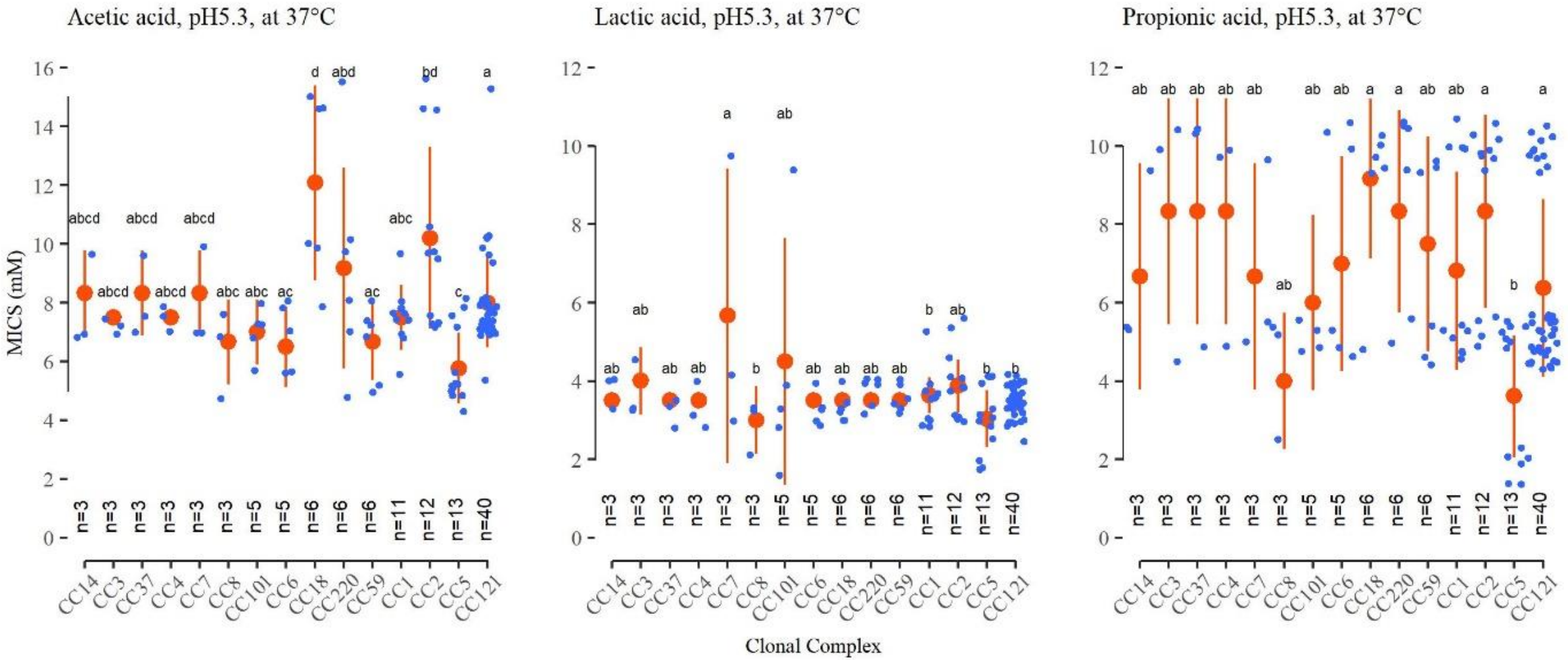
Strain variation in organic acid tolerance (*n* = 150) (Acetic, Lactic & Propionic acid)

Strain variation in ability to form biofilm (*n* = 150)

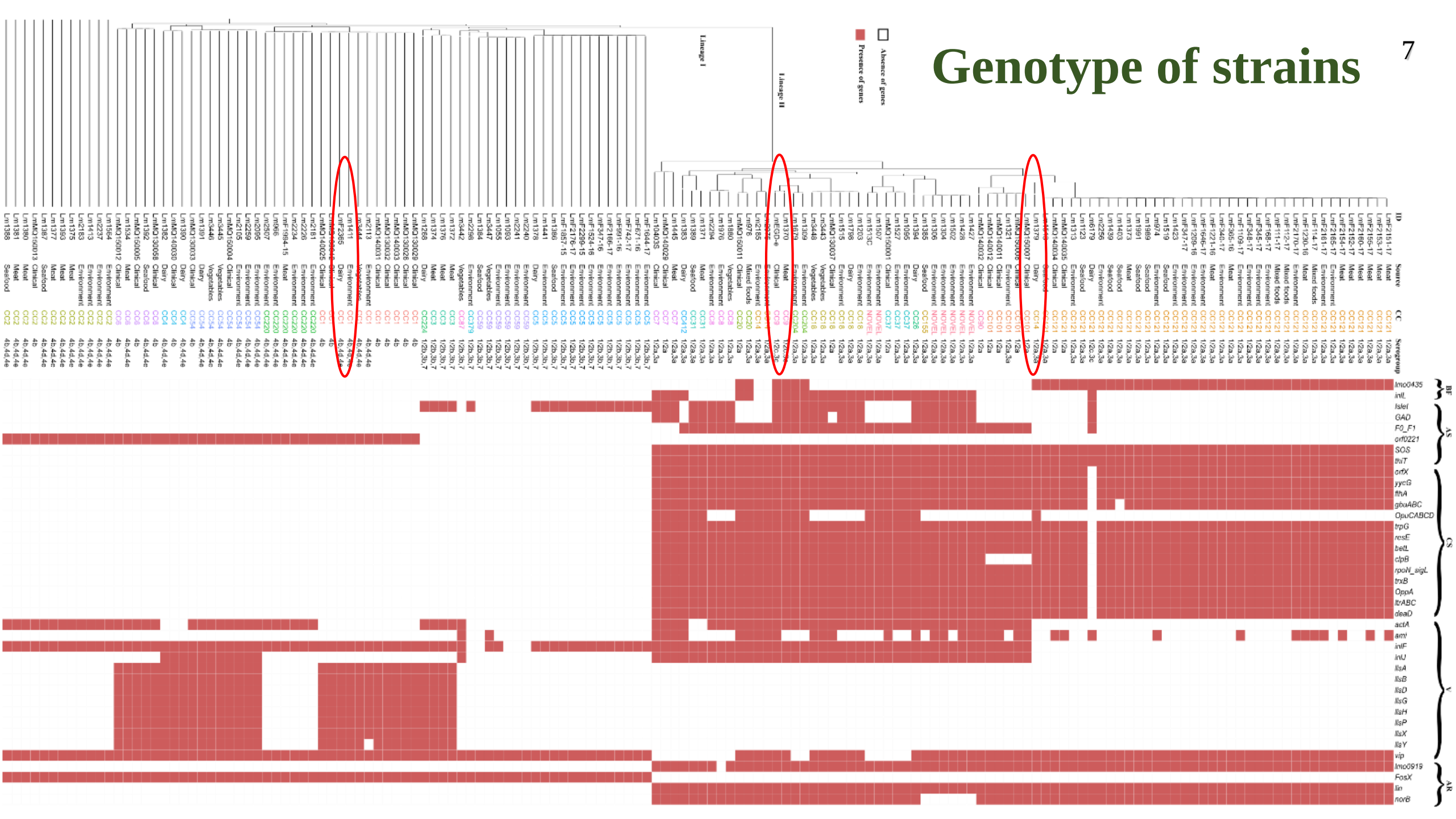
Pan-genome based comparative genomic of *Lm* (*n* = 150) (cold tolerance, acid tolerance, ability to form biofilm and presence of virulence genes).

Challenge study to determine maximum growth rate of *Lm* (cheese)

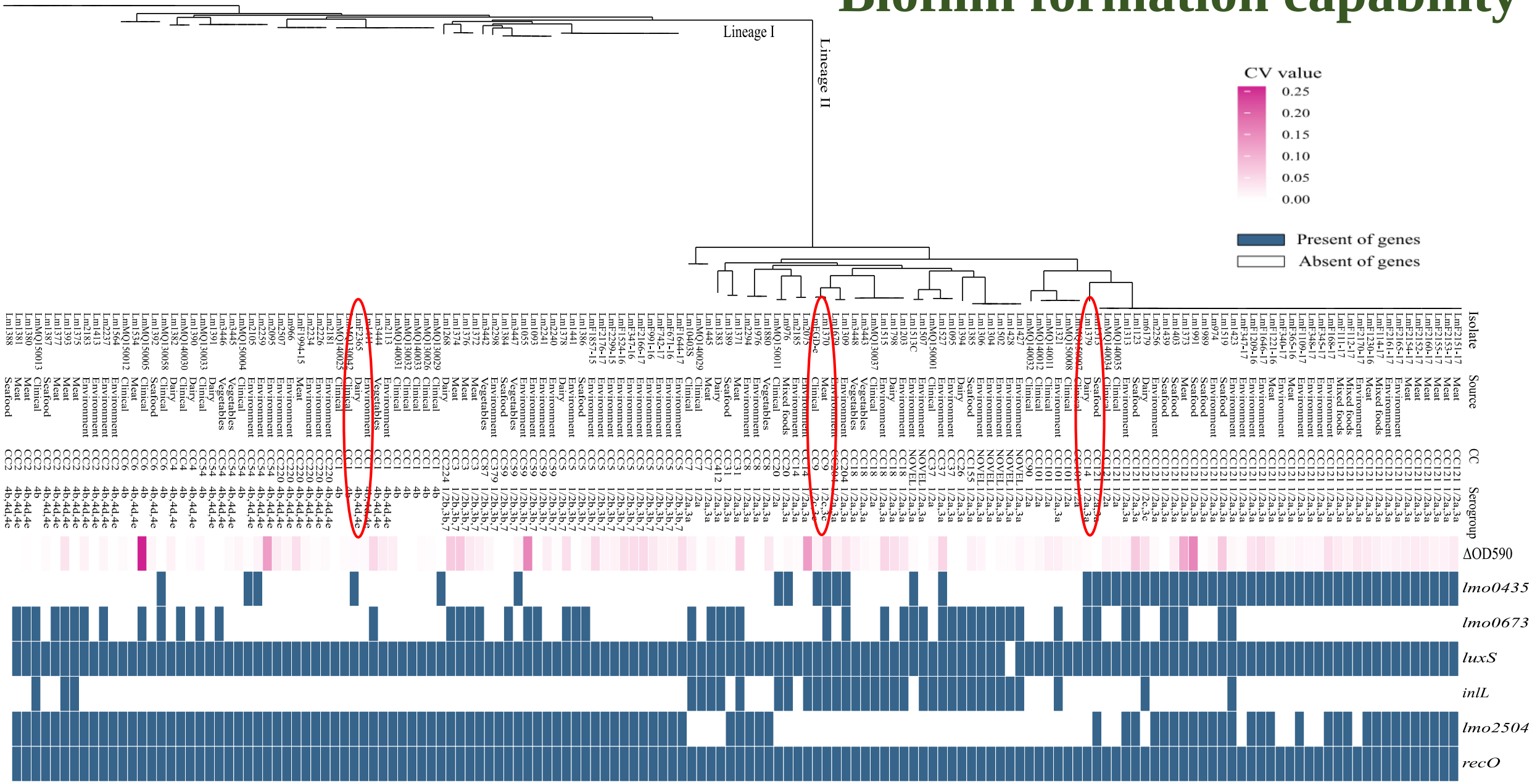
Organic acid tolerance among strains



Genotype of strains

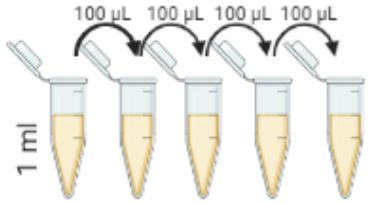


Biofilm formation capability



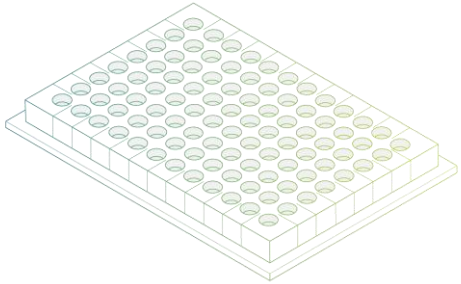
Temperature growth screening of 177 strains

Overnight



Standardised and diluted to 10^2 CFU/mL

Transfer to microtiter plate



5 µL per spot 8 spot per strain

Incubate at 4, 10, 15, 20 and 30°C

Detect colony appearance twice a day

Time a colony needed to appear on the agar surface after inoculation

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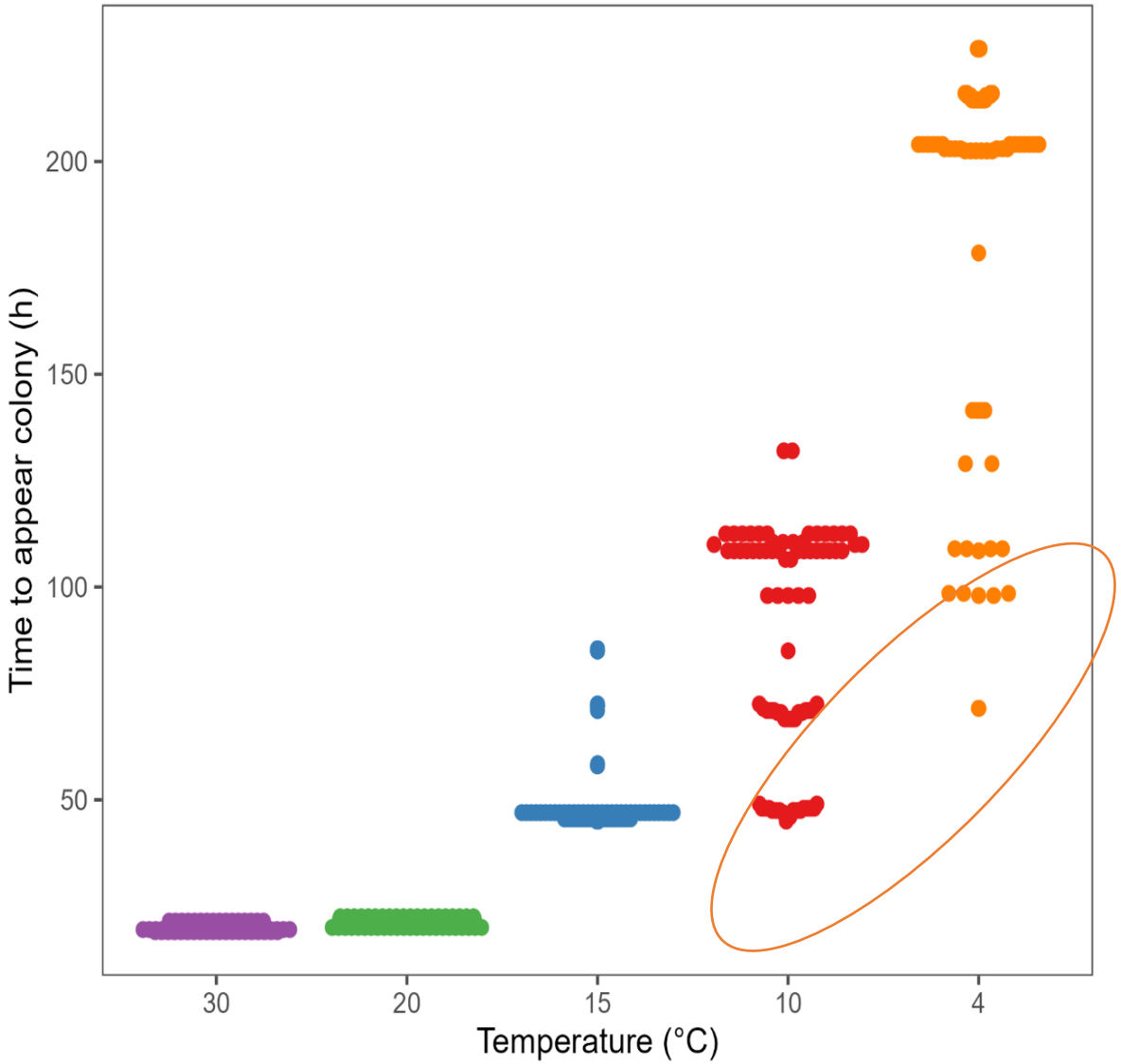
Selection of strains for challenge studies



Euclidean distance & Ward H.Cluster

Strains

954

12MOB079LM

F2365

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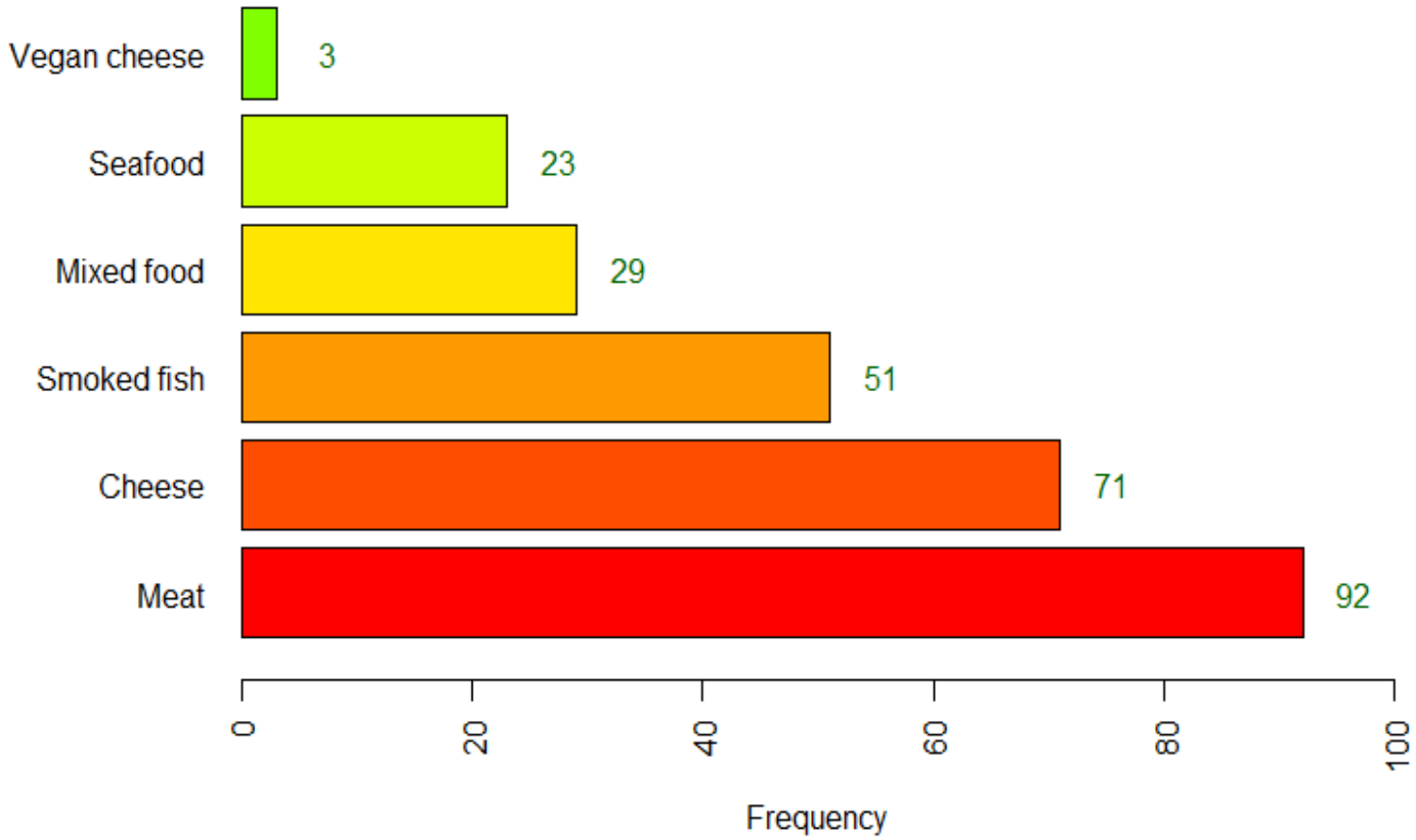
Strains characteristics

F2365	F2365 (UCC) Dairy outbreak isolate CC-1, 4b, lineage I	Low organic acid tolerance (Myintzaw *et al*., 2022a) High pH and Mild NaCl tolerance (Wu *et al*., 2022) Slow growth 4 & 7 °C (Myintzaw *et al*., 2022b)
954	954 (Teagasc Moorepark) Dairy environment isolate CC-9, 1/2c, lineage II	Mild organic acid tolerance (Myintzaw *et al*., 2022a) Low pH and Low NaCl tolerance (Wu *et al*., 2022)
1379	12MOB079LM (EURL) Dairy food isolate CC-14, 1/2a,3a, lineage II	Mild organic acid tolerance (Myintzaw *et al*., 2022a) High pH and Low NaCl tolerance (Wu *et al*., 2022) Fast growth at 4 & 7 °C (Myintzaw *et al*., 2022b)

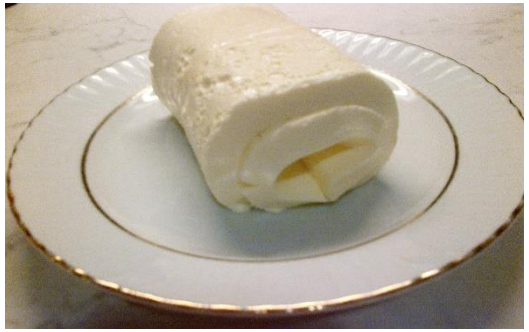
Lm incident in RASFF (RTE food)



Number of serious alerts by food type for *L. monocytogenes*-related in the RASFF(2018 March—2023 March)

Recent *Lm* incident related to cheese

- One person in the **UK** has died in a *Listeria* outbreak link to soft cheese. **Mar 24, 2023**
- Outbreaks of Listeriosis Linked to Fresh, Soft Queso Fresco-Type Cheeses in the **U.S**. **February 6, 2023**
- *Listeria* outbreak from deli meat and cheese kills one, sickens dozen in **US**. **November 9, 2022**
- Two people have died and 33 sicken in a *Listeria* outbreak in **Italy** that could be linked to cheese. **August 23, 2022**
- Three dead in **Austria** *Listeria* outbreak that could be linked to Kajmak cheese. **September 20, 2022**



Challenge studies in semi soft, rind ripened cheese

Milk: Whole Cow's milk, pasteurised

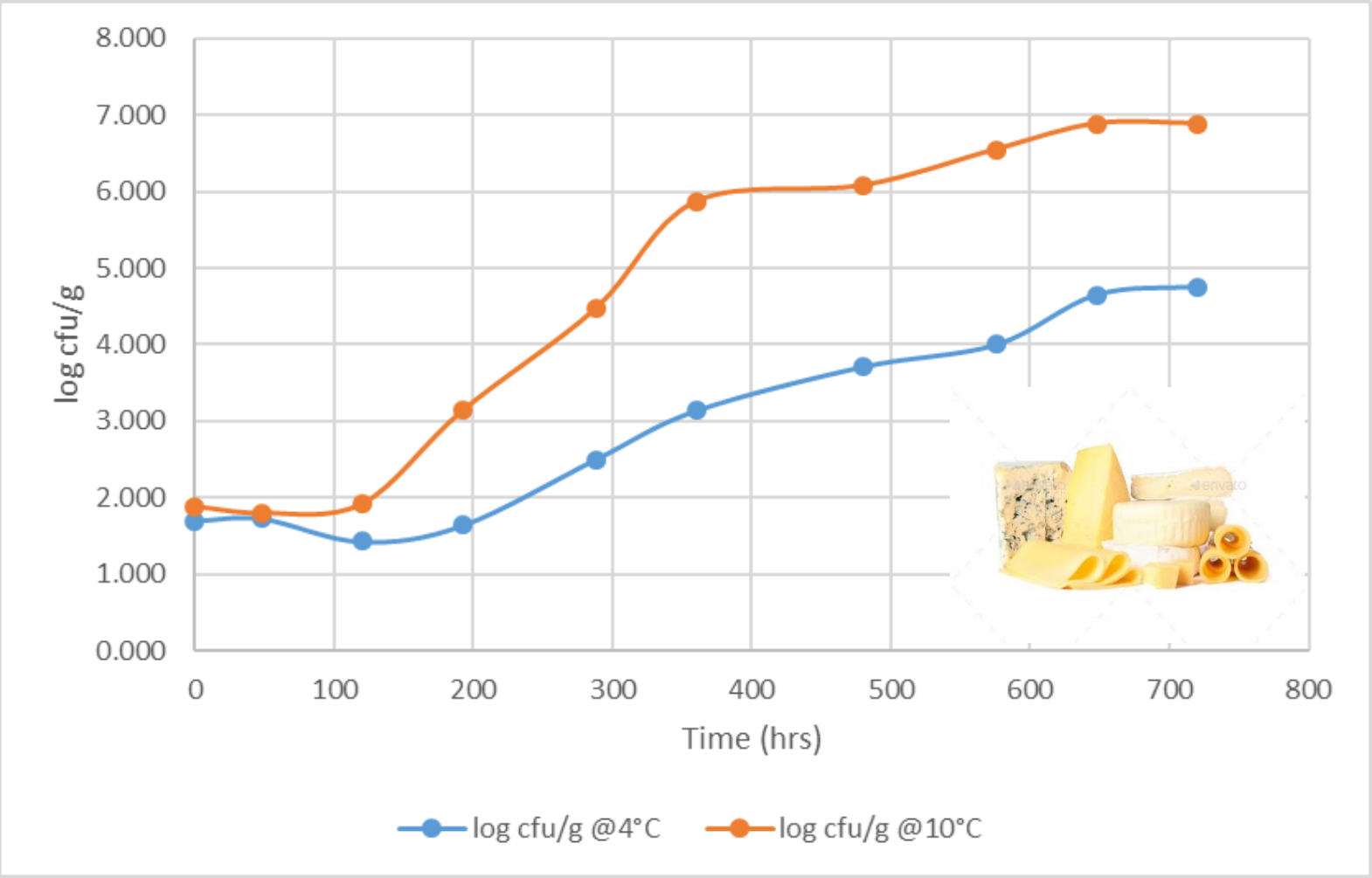
Type: Semi soft, rind ripened

Ready for eating: from 10 days

Shelf life: 28 days

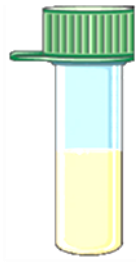
Physiochemical property	
pH	5.93±0.15 (*n* = 18)
a_w	0.96±0.01 (*n* = 18)
Salt (%)	1.82±0.10 (*n* = 18)

Preliminary challenge studies in cheese



1379 (EURL strain) from dairy

Challenge study according to EURL *Lm* guideline



Stress adaptation
10ºC – 3 days
4ºC – 12 days

Standardised, diluted and inoculate 100 cfu/g 1% wt of sample. 16 test units

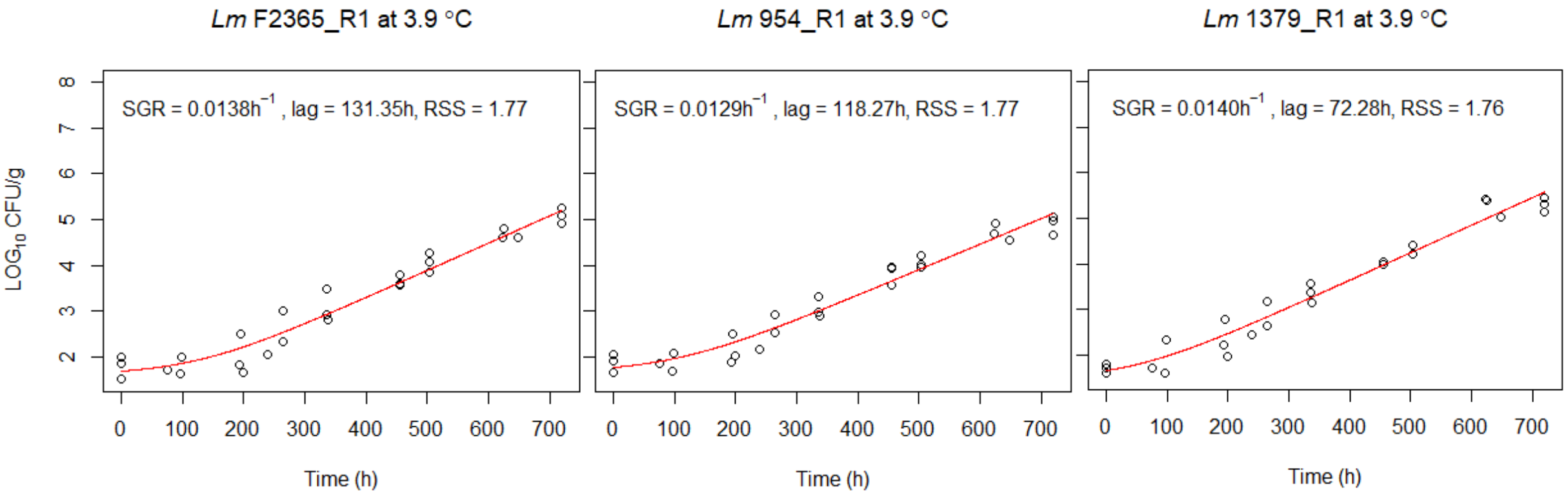
Incubate and enumerate

Detection of *Lm* at T_0

TBC at T_0 and T_{end}

Measurement of a_w, pH, Salt at T_0 and T_{end}

Growth at Static Refrigeration Temperature



ComBase prediction: SGR = 0.007/h, Lag = 241.96 h, N_{max} at 720 h = 5.06 Log_{10}CFU/g

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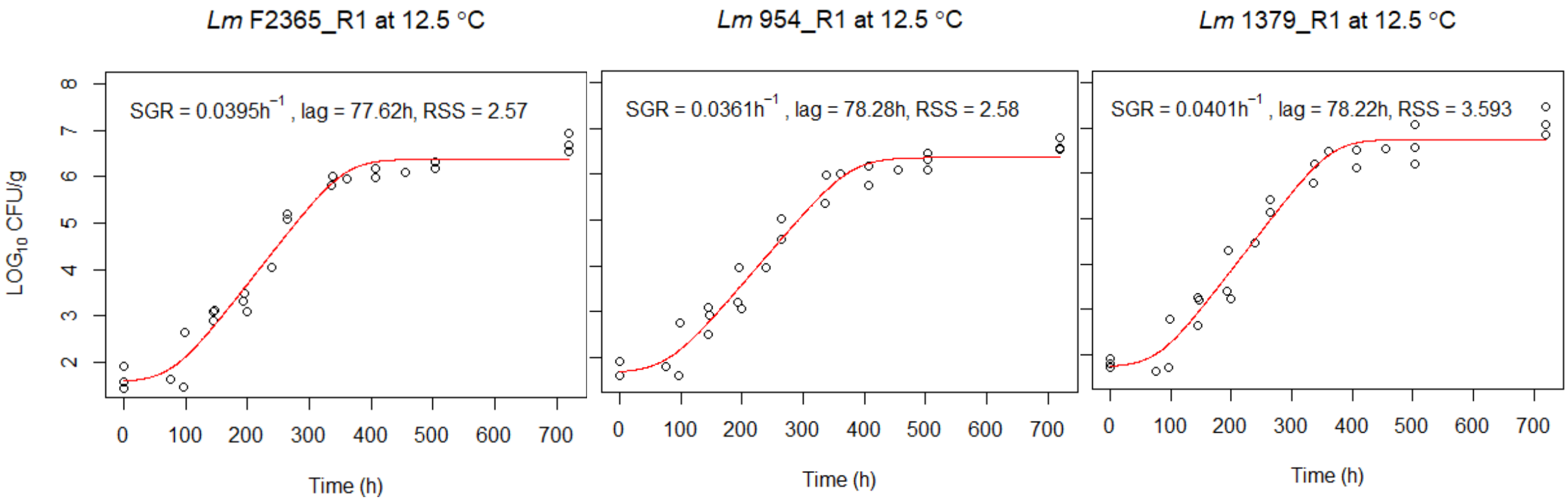
Growth at Static Abuse Temperature



ComBase prediction: SGR = 0.033/h, Lag = 51.33h, N_{max} at 720 h = 8.52 Log_{10}CFU/g

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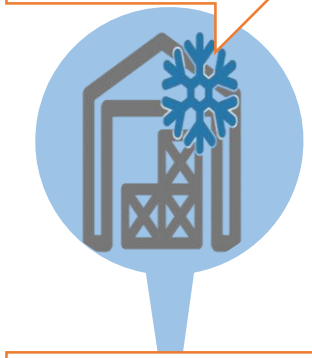
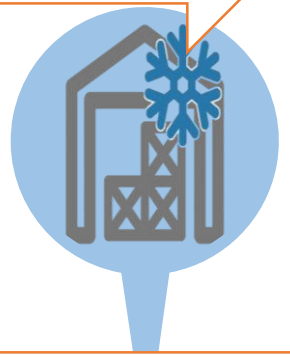
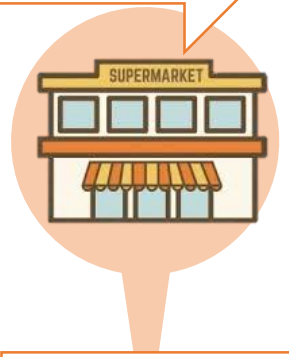
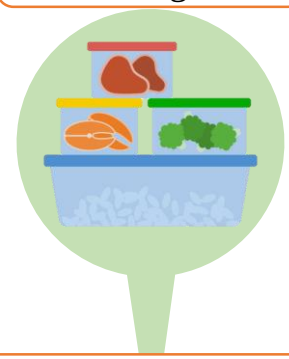
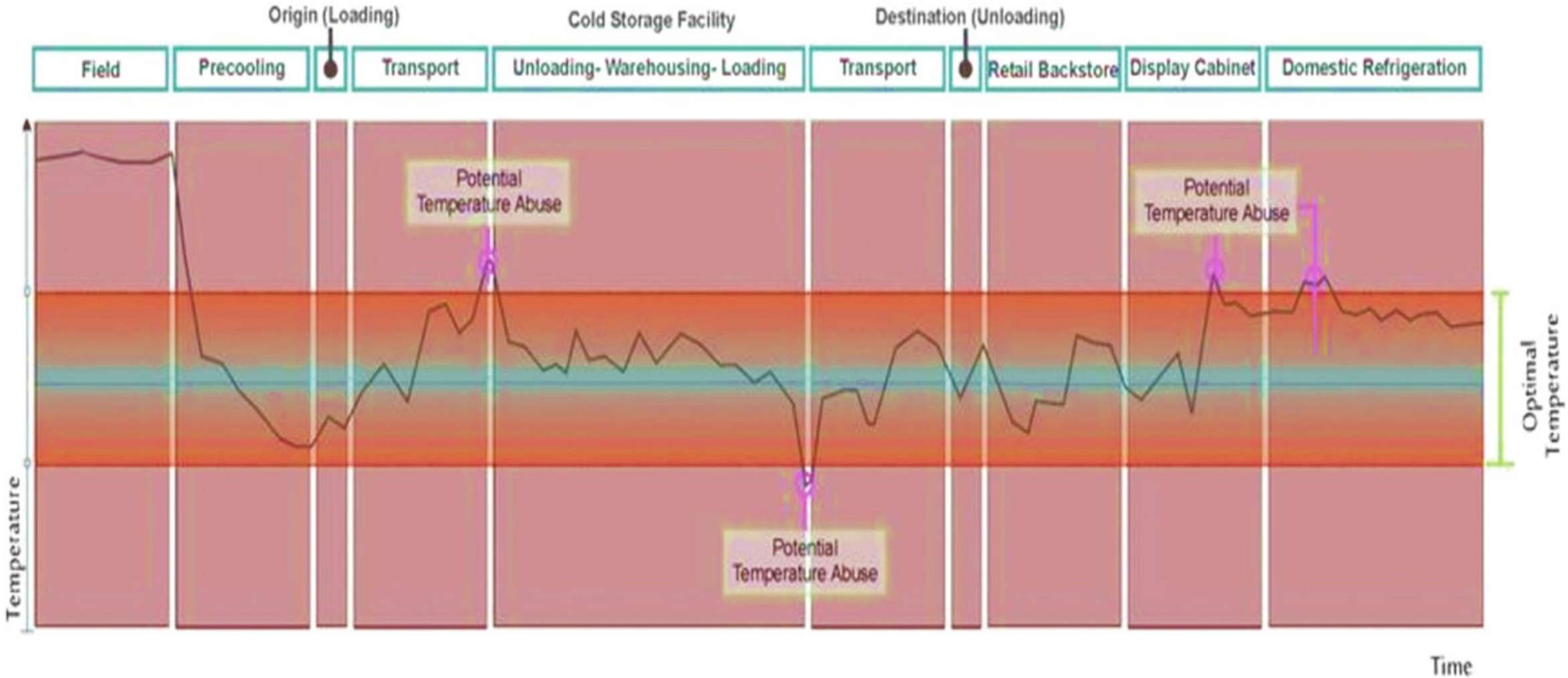
Note: on Growth at Static Temperature

- Growth did not reach stationary phase at either refrigeration or abuse temperature.
- Baranyi and Roberts, 1994, without N_{max} and with N_{max} for refrigeration and abuse conditions were fitted to investigate growth parameters.
- Strain-to-strain variation in growth was observed in both conditions.
- Each of the selected strains grows better in this cheese product than predicted by ComBase which was predicted in broth.

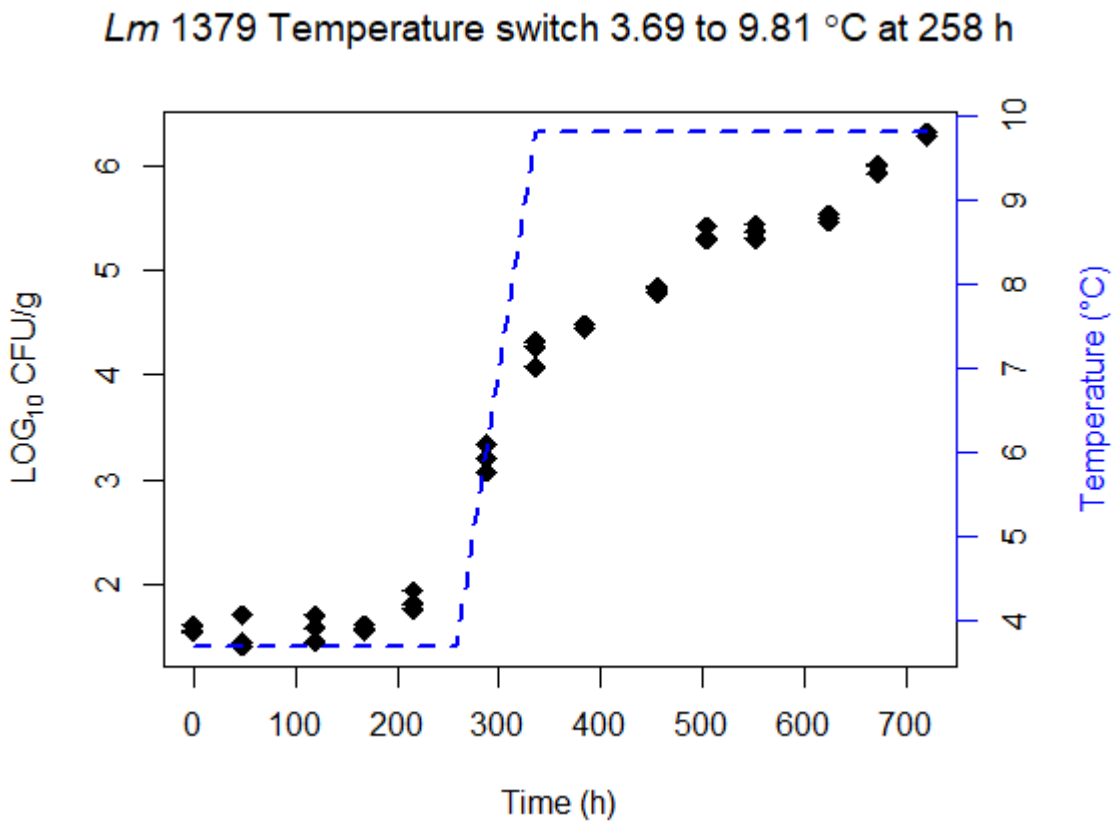
Temperature along food chain

Processor storage	Transport to distribution	Distribution centre storage	Transport to retail	Retail storage	Consumer shopping	Consumer storage
						
Canada 2.19—6.10 °C (McKellar et al., 2014)	Canada 1.17—7.91 °C (McKellar et al., 2014)	Canada 1.36—2.56 °C (McKellar et al., 2014)	Canada 1.99—8.47 °C (McKellar et al., 2014) Japan 5-7 °C (Ndraha et al., 2018)	Canada 3.42—5.11 °C (McKellar et al., 2014) Spain 4—8 °C France 0—7 °C Slovenia 2—6 °C USA 0.6–15.4 °C (Ndraha et al., 2018)	?	Greek 7—17.9 °C (Andritsos et al., 2021) Japan 0—18 °C (Mori et al 2020) Spain 3.6—10.4 °C (Jofré et al., 2019) Europe 5.3—11.7°C (Bonanno & Bergis, 2021)

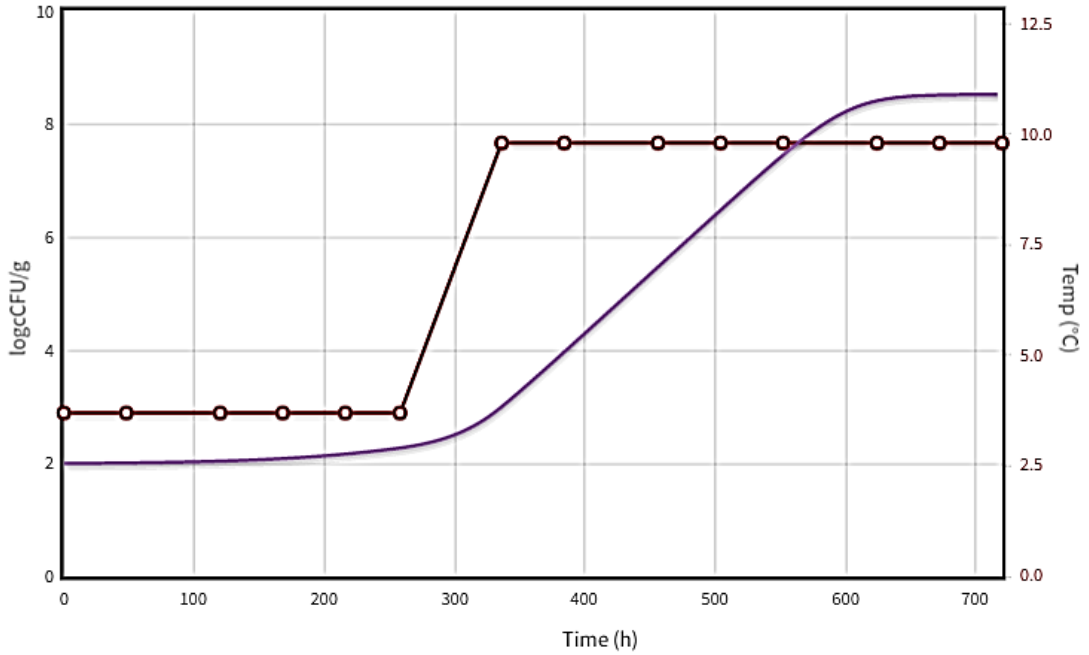
Potential temperature abuse (Mercier et al., 2017).



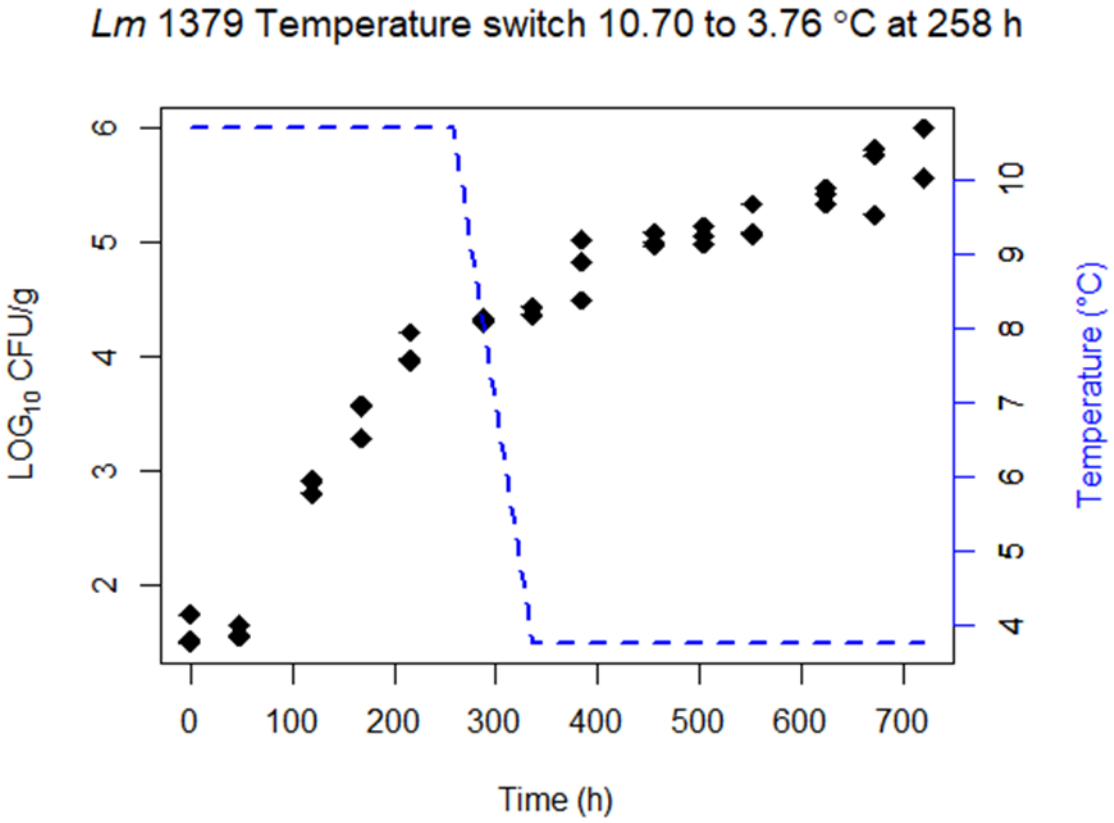
Growth with Dynamic Temperature Shift 4°C to 10°C



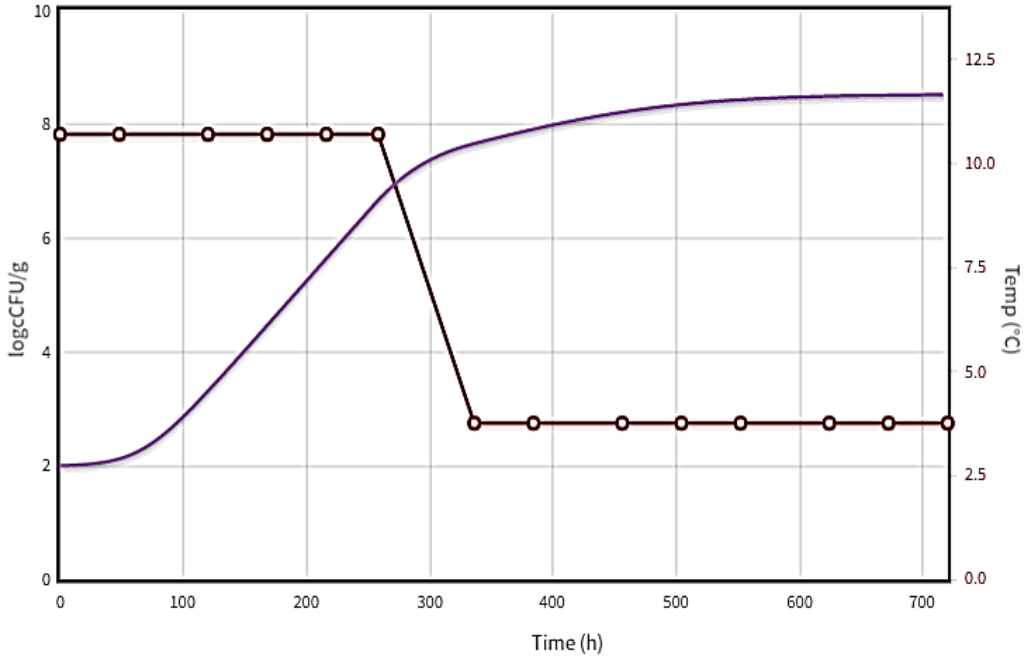
ComBase prediction for same condition



Growth with Dynamic Temperature Shift 10°C to 4°C



ComBase prediction for same condition



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To Conclude

- The capability of *L. monocytogenes* to proliferate in semi soft, rind ripened cheese in the food chain at a cold chain and abuse temperatures was highlighted.
- Before the end of shelf life, *L. monocytogenes* reaches levels likely to be harmful to the consumer.
- The development of a predictive model for dynamic temperature shifts to describe the contamination level in RTE food like cheese is crucial in assessing the risks of listeriosis accurately.

Publication arisen form the project

foods

MDPI

Article

Omnibus Modeling of *Listeria monocytogenes* Growth Rates at Low Temperatures

Vincenzo Pennone [1], Ursula Gonzales-Barron [2], Kevin Hunt [3], Vasco Cadavez [2], Olivia McAuliffe [1] and Francis Butler [3,*]

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Correlation of organic acid tolerance and genotypic characteristics of *Listeria monocytogenes* food and clinical isolates

Peter Myintzaw [a], Vincenzo Pennone [b], Olivia McAuliffe [b], Máire Begley [a], Michael Callanan [a,*]

microorganisms

MDPI

Article

Variability in Cold Tolerance of Food and Clinical *Listeria monocytogenes* Isolates

Peter Myintzaw [1], Vincenzo Pennone [2], Olivia McAuliffe [2], Máire Begley [1] and Michael Callanan [1,*]

Acknowledgment

- Special thanks to PI Prof: **Olivia McAuliffe**.
- Thanks to **Dr. Kieran Jordan & Dr Triona O'Brien** who gathered and maintain the *Lm* collection since 1984.
- Thanks to former Postdoc **Dr Vincenzo Pennone** for sanitizer and cold tolerance screening and preliminary cold growth study of the collection.
- Thanks to former Postdoc **Dr Mairead Holton** for preliminary growth study in cheese.

Thank you for your attention and any question?

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