How to validate in a variable world: Use Data, Lots of Data, Both from Experiments and from Literature

Marcel Zwietering Laboratory of Food Microbiology

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Session: Use of Predictive Microbiology for Process Validation Encompassing Biological Variability

Zero risk – Control – Variability – Heterogeneity - Biology

For food safety we want to be absolutely sure!

But absolute does not exist;

Food products, microorganisms, and humans are biological entities

Variability is inherent in biology

Also technical parameters show variability .....

Complexity ..... difficult decisions .....

Zero risk – Control – Variability – Heterogeneity - Biology

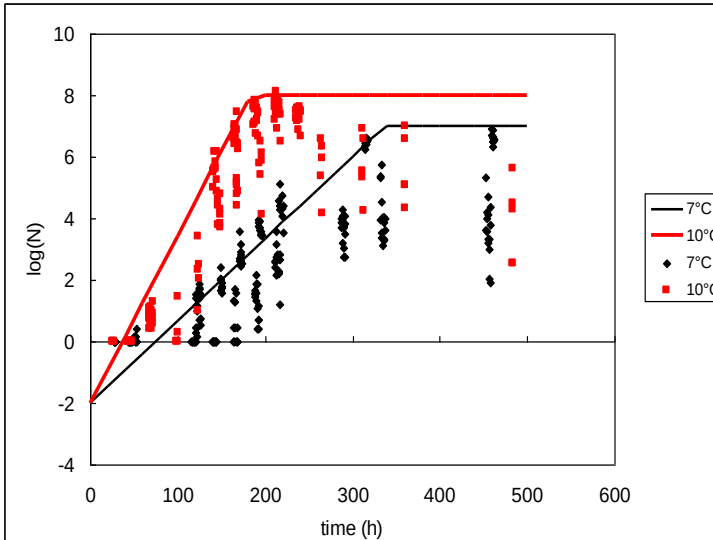
CONSUMERS, THE GOVERNMENT, THE INDUSTRY:

ZERO RISK in wonderland....

CONTROL :: verification :: out of control
validation :: safe by design

BIOLOGY

VARIABILITY (HETEROGENEITY)



How is Food Safety organised ?

Verification by MicroCrit

Monitor Critical Limits

Validated CCPs

HACCP

PRP (GMP, GHP, ...)

Validation

- **Monitoring:** a planned sequence of observations of control parameters to assess whether a control measure is under control.
- **Verification:** The application of procedures and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.
- **Validation:** Obtaining evidence that a control measure, if properly implemented, is capable of controlling the hazard to a specified outcome

Validation

- Experimental evidence
  - challenge testing
    - lab scale testing with pathogen
    - in process: indicators or surrogates
- Quantitative microbiology
    - representativity
    - variability
    - uncertainty
    - databases
    - meta-analysis

The FSO concept:

primary production

H_o-ΣR+ΣG+ΣC <PO performance objective

food industry

H_o-ΣR+ΣG+ΣC <PO

PC: performance criteria (6D)

consumer

H_o-ΣR+ΣG+ΣC <FSO

process/product criteria (71.5°C, 16.6s)

forces to quantify !

forces to look over the whole chain !

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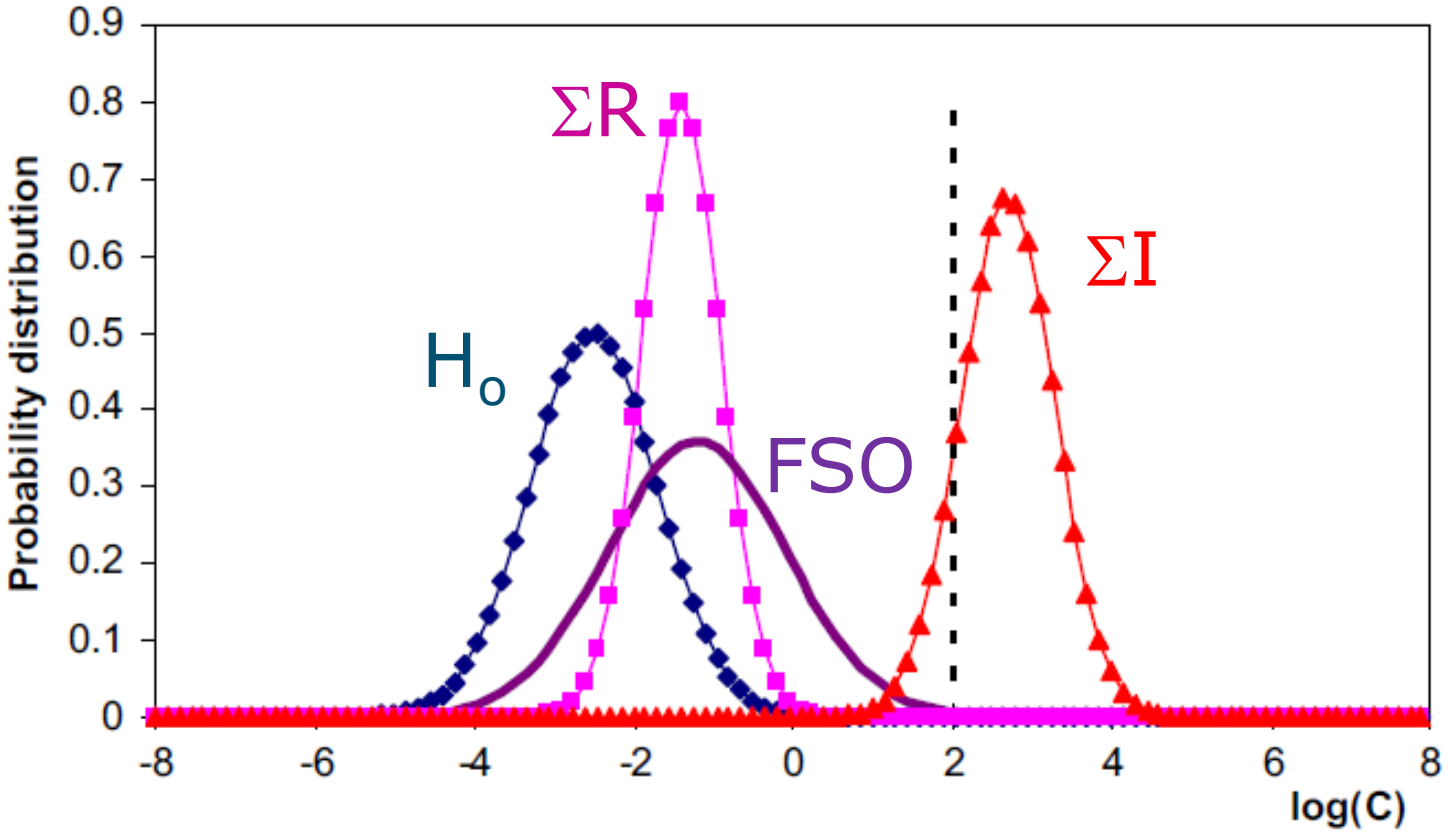
Validation of control measures in a food chain using the FSO concept

M.H. Zwietering [a,*], C.M. Stewart [b], R.C. Whiting [c], International Commission on Microbiological Specifications for Foods (ICMSF)



Probability distributions of **H_0 – ΣR + ΣI = FSO**

of *Listeria monocytogenes* in fresh cut lettuce (0.2% above the FSO)

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Forces to also include variability of all factors

Need to quantify effects of all factors and all variabilities

Microsoft Excel - stochasticFSOtool01_02.xls

	meanlog	sigma	FSO	P(x>FSO)	%	P(x<FSO)	%
overall	-1.2	1.11269942	2	0.002014457	0.201446	0.997986	99.79855

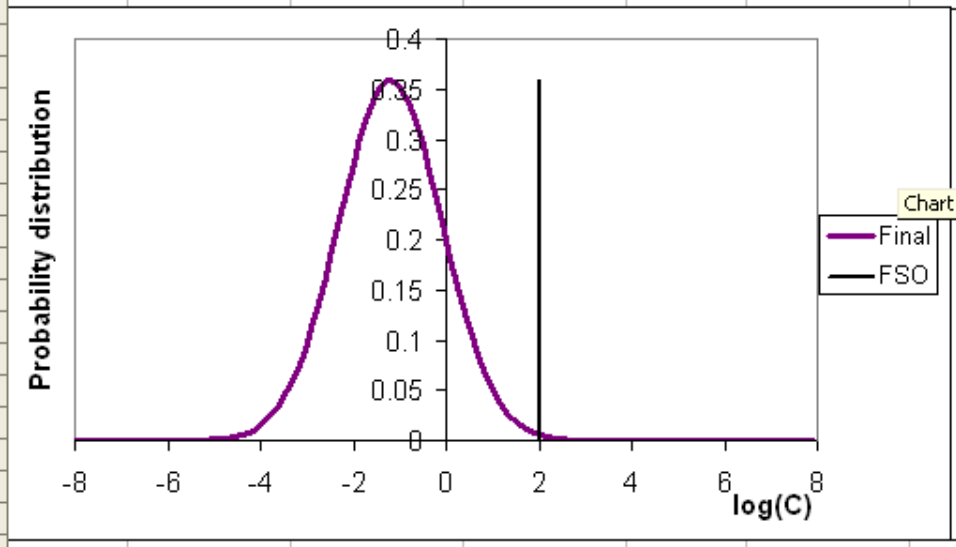


Fig. 1: Final distribution in relation to FSO

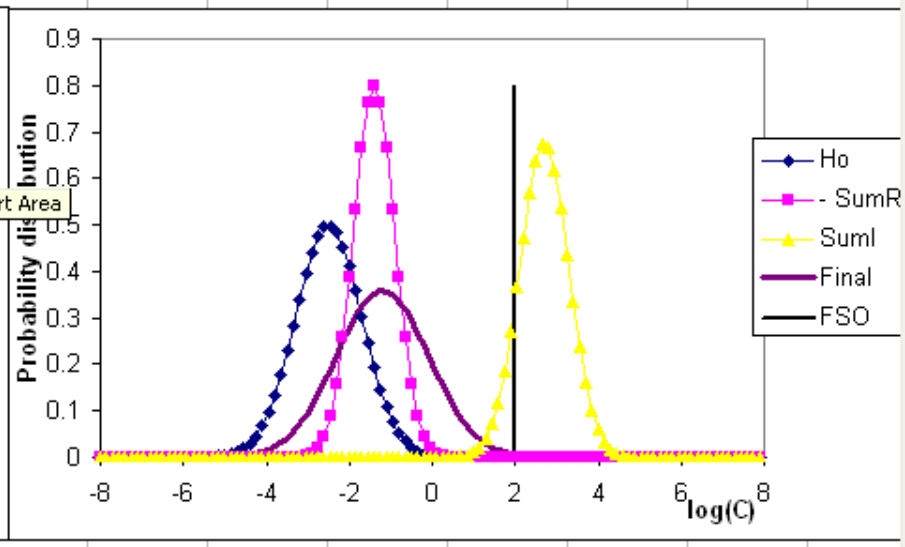


Fig. 2: Various underlying distributions resulting in the final exposure distrib

manual | single stage | multiple | equality

Ready

Sources of variability / heterogeneity

Experimental error
Biological variability
Cell history, physiological state
Genetic variability
Strain variability

Product specific effects

Variability in controlling factors, environment, humans, etc

Microbe

Product

Process and chain

QUANTIFY: REALISTIC PREDICTIONS
DETERMINE SOURCES
RANK IMPORTANCE
CONTROL WHERE POSSIBLE
BIOLOGICAL INSIGHT

Dilemma

We need Data, Lots of Data,

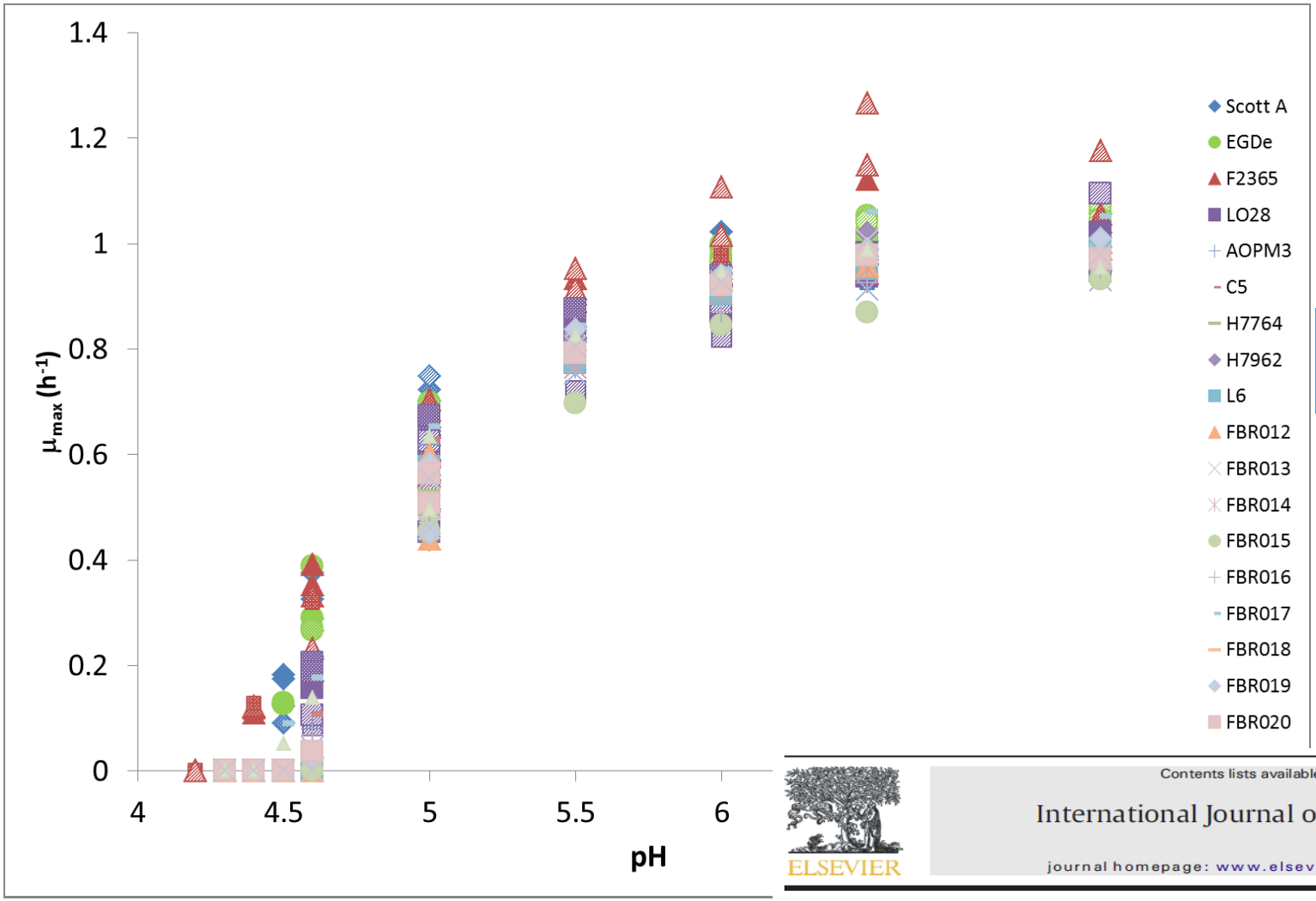
of levels, growth, inactivation and recontamination

Experiments

- Model experiments
- Surrogates in plants

- Strain selection / cocktail / prehistory (NOT 37 °C)

Variability in Growth rate(pH) *Listeria*



E

B

S

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Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

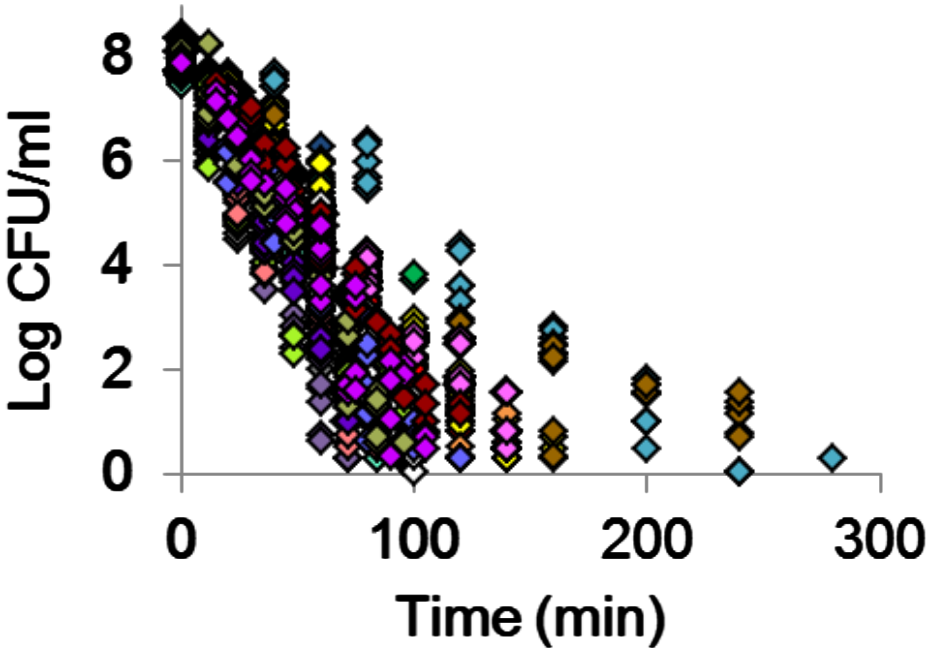
ELSEVIER

Research paper

Quantifying strain variability in modeling growth of *Listeria monocytogenes*

D.C. Aryani [a,b], H.M.W. den Besten [b,*], W.C. Hazeleger [b], M.H. Zwietering [a,b]

Variability in inactivation rate



- Experimental
- Biological
- Strain

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Quantifying variability on thermal resistance of *Listeria monocytogenes*

D.C. Aryani [a,b], H.M.W. den Besten [a,b,*], W.C. Hazeleger [b], M.H. Zwietering [a,b]

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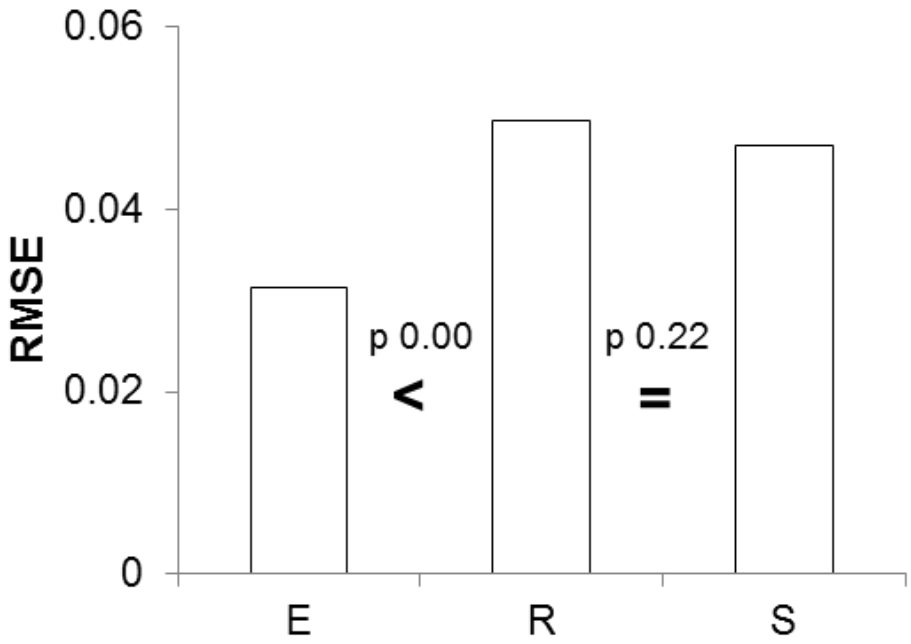
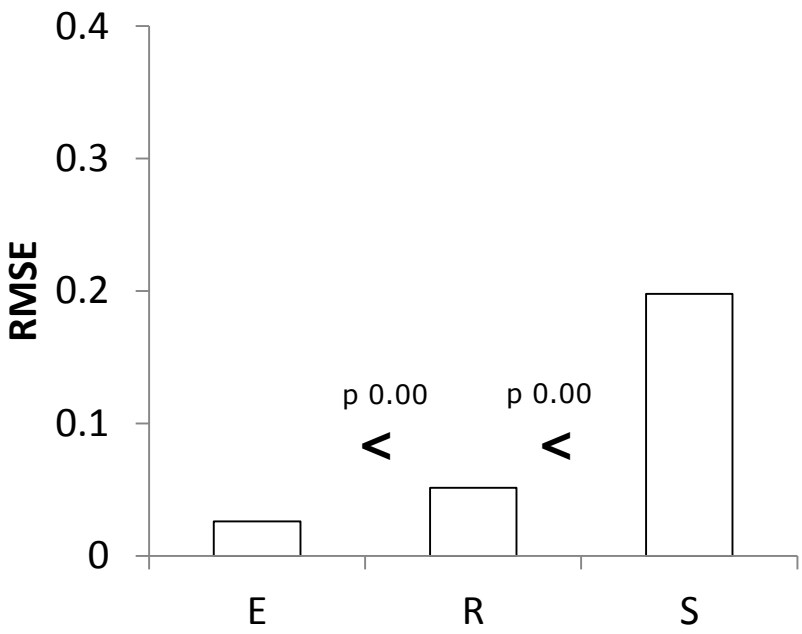
Quantifying Variability in *Listeria*: μ and D

μ_{max} pH



D-value



S = R > E

500-1000 points per variable

S >> R > E

360 D values

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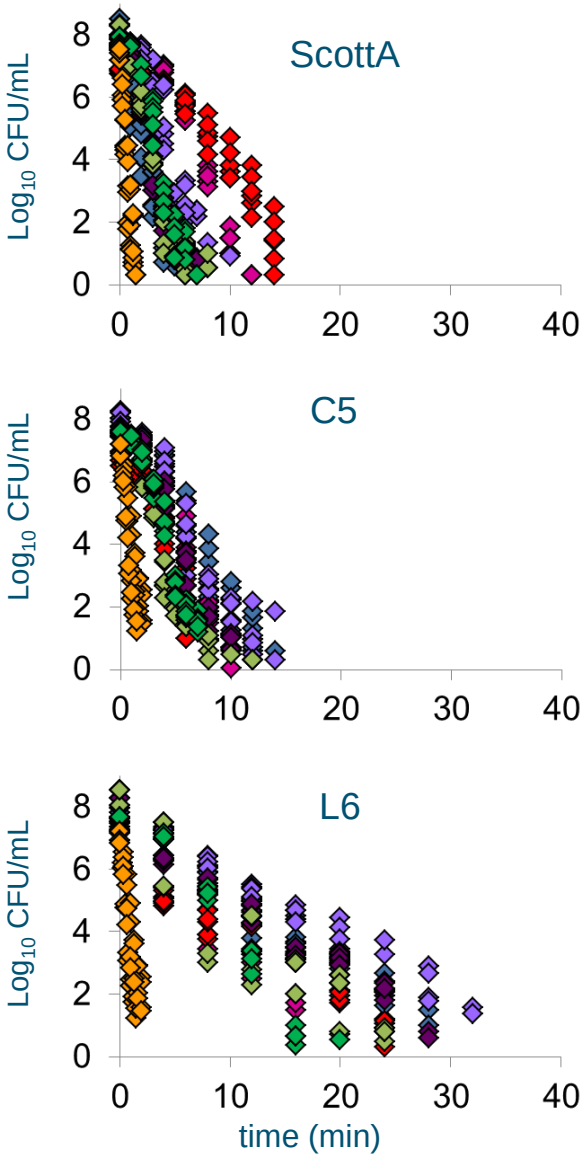
Impact of growth history



stationary
pH 6
pH 5
NaCl 2.5%
NaCl 5%
7°C
15°C
exponential

All show effect but “exponential” is biggest and consistent

All variabilities are equal but some are more equal than others

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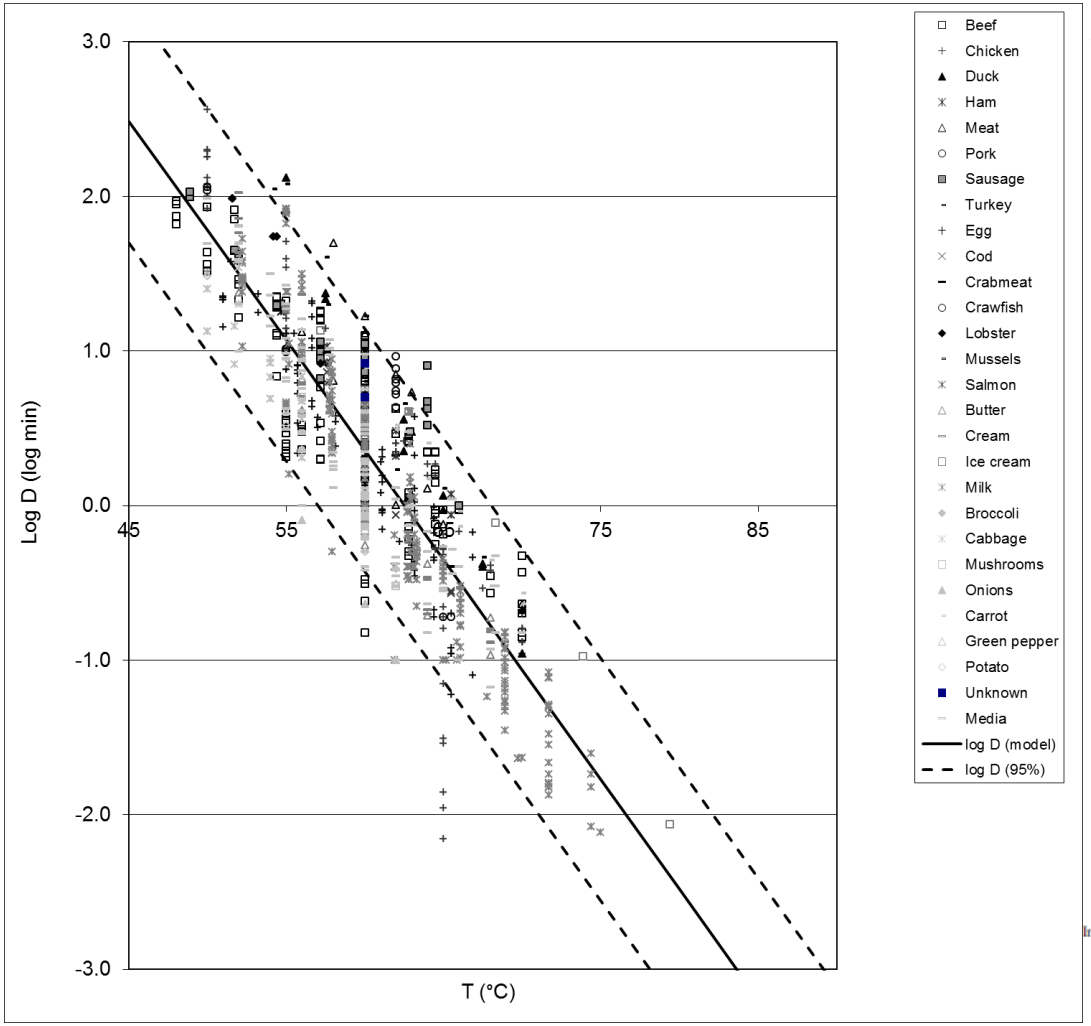
Microbial variability in growth and heat resistance of a pathogen and a spoiler: All variabilities are equal but some are more equal than others

Heidy M.W. den Besten [b,*], Diah C. Aryani [a,b], Karin I. Metselaar [a,b], Marcel H. Zwietering [a,b]

Benchmarking: meta-analysis all strains, products, years, labs, history....



940 D values

Available online at www.sciencedirect.com
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International Journal of Food Microbiology 107 (2006) 73 – 82
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

A systematic approach to determine global thermal inactivation parameters for various food pathogens

Esther D. van Asselt [1], Marcel H. Zwietering *

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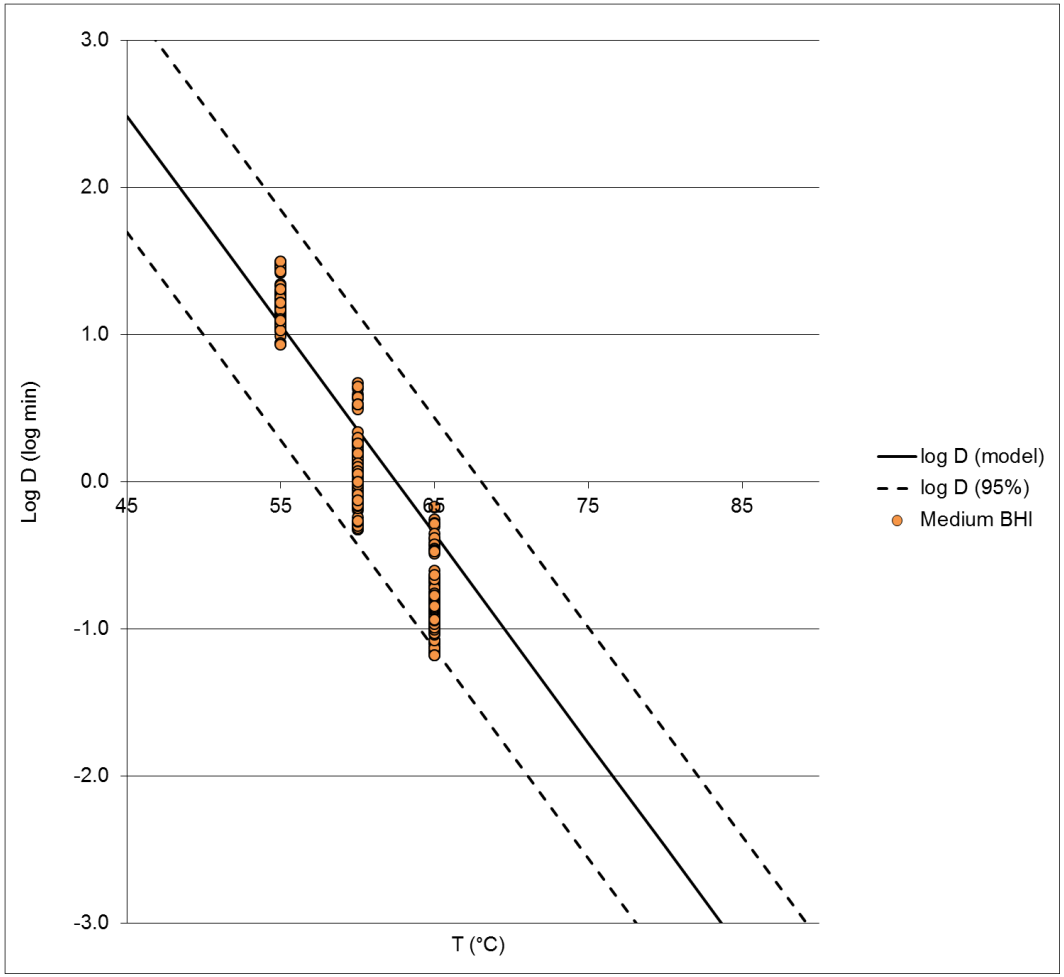
Benchmarking with strain variability



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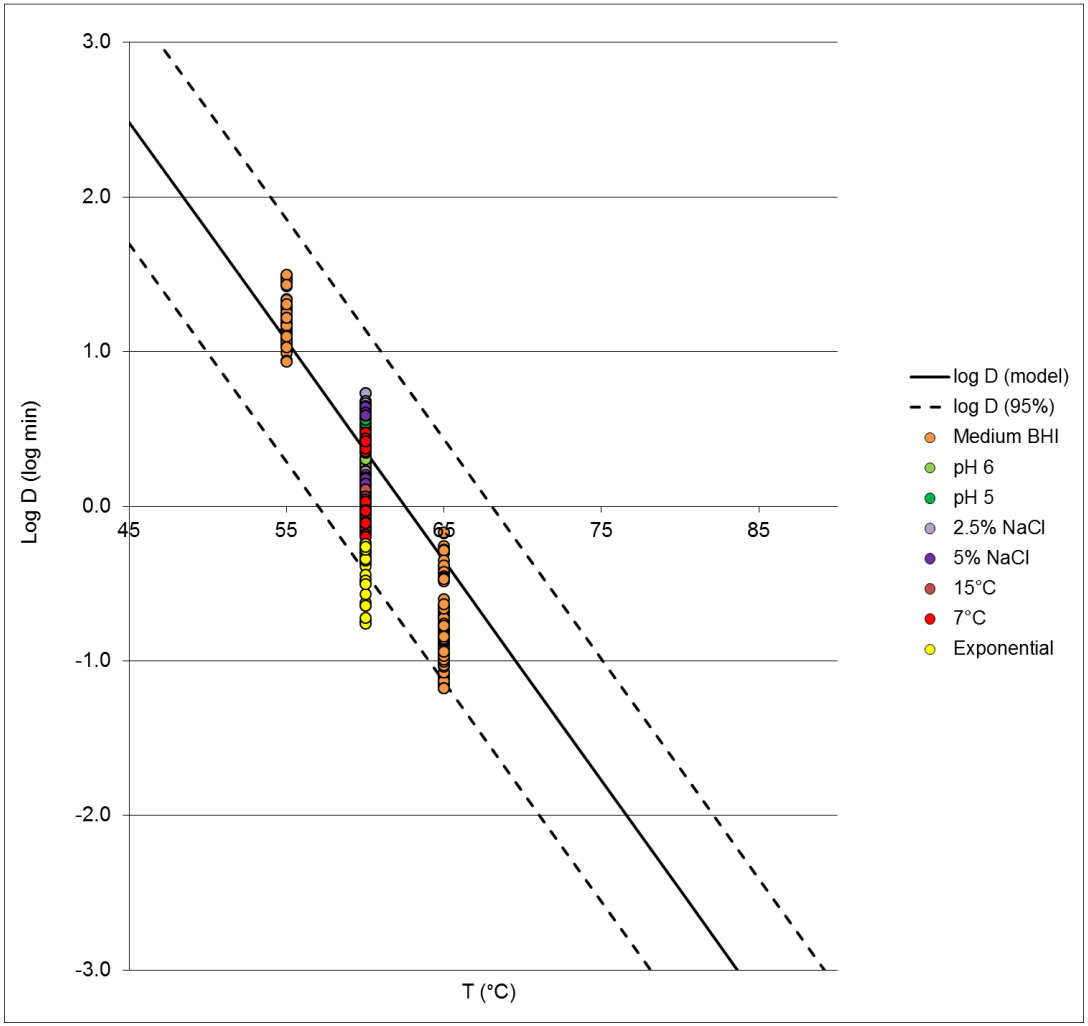
Benchmarking



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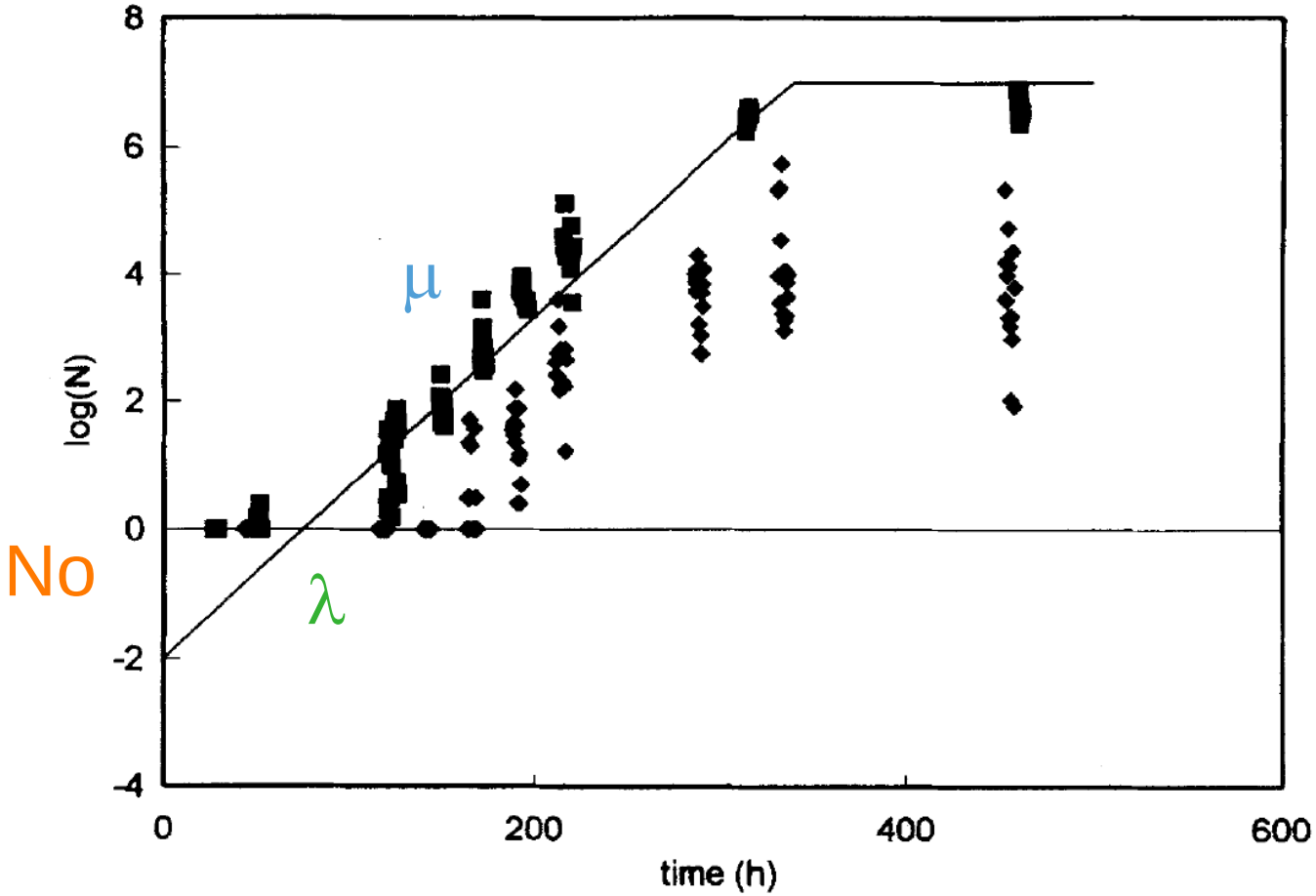
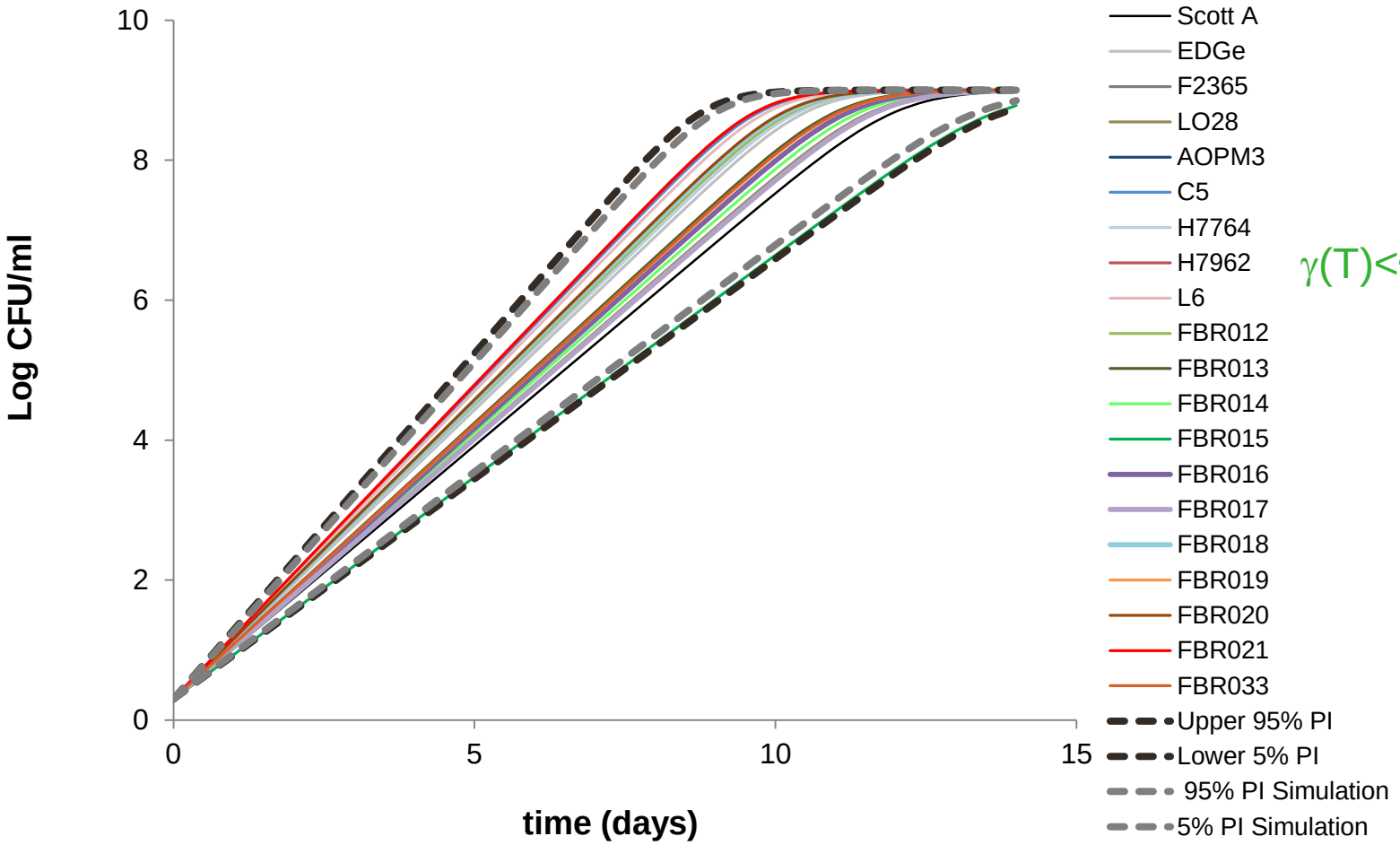
Variability in level at constant temperature



Comparison of the predicted and measured number of organisms in milk stored at 7 °C from two labs: ■ ◆

Growth of *L. monocytogenes* strains in milk

pH = 6.6 T = 7°C a_w=0.993



$\gamma(T)<\gamma(a_w)<\gamma(pH)$

μ_{ref} = 0.99 (h^{-1}); γ(pH) = 0.98; γ(T) = 0.082;
$\gamma(a_w)$ = 0.96; γ (total)= 0.077

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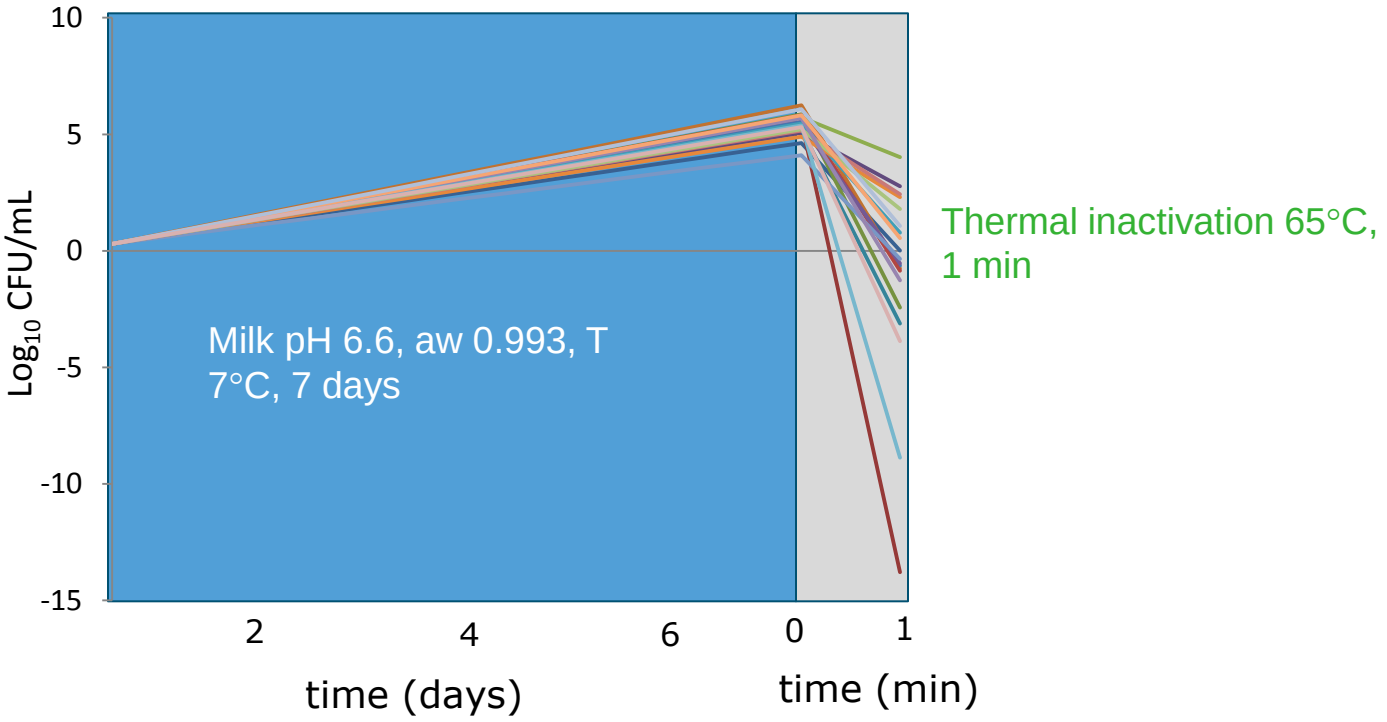
The Effect of Strain Variability on Growth and Thermal Inactivation



both show large variability but inactivation shows biggest effect

..... some are more equal than others

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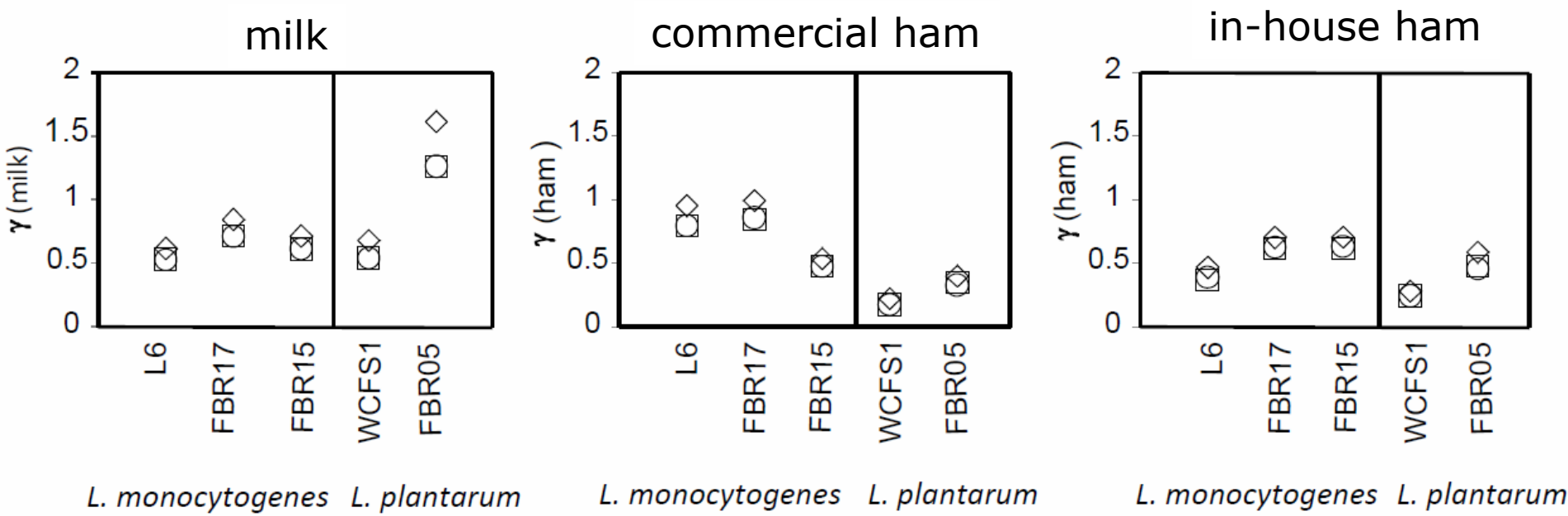
Validation in milk and ham



◊ : Gompertz

□ : Logistic

O : Baranyi

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Data Sources

- Data sources:

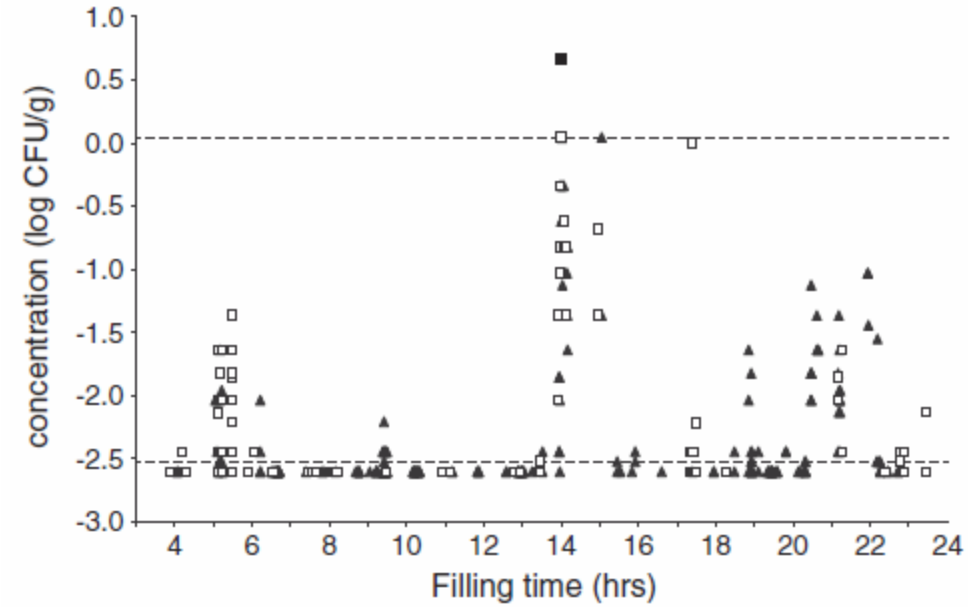
+ literature data
+ databases
+ historical data
+ experimental data
+ storage tests
+ challenge tests
+ predictive models
+ safe harbours
+ basic knowledge
+ logic

- criticism

weak and their strong points

Also other source than biological variability

- Initial levels
- Storage time and T
- Product characteristics
- Recontamination



415 MPN, 2290 plate counts

- Balanced focus on all variables and their variabilities
- Too much focus on growth and inactivation ?

Data Sources

- Models and databases
- Accurate models do not exist
- It are only realistic models: and they include large variability
- Only if one knows well all variables, including quantified variability can one assess the realistic ranges. However the selection of main effects in this respect is the crucial challenge

Variability and percentiles

- What if we use the 95 percentile, or the 99th ?
- In a batch with 100.000 product units ?
- With 10.000 batches produces per year ?
- But is there are 5 factors at their 99th percentile it is just 1 out of 10.000.000.000
- But only if these 5 are equally important. If one is mainly determinant, it is still 1 out of 100 !
- What is fail safe ???

Thanks

Enjoy variability in life

Then it becomes valid !