Microbiological variability

Sources and implications for food safety and spoilage

Heidy den Besten

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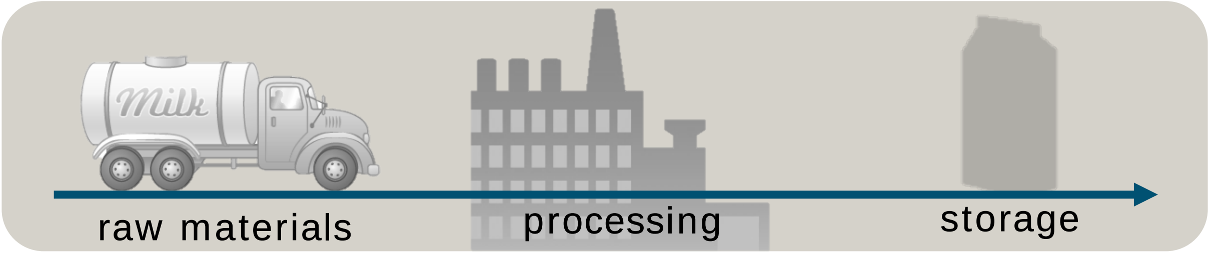
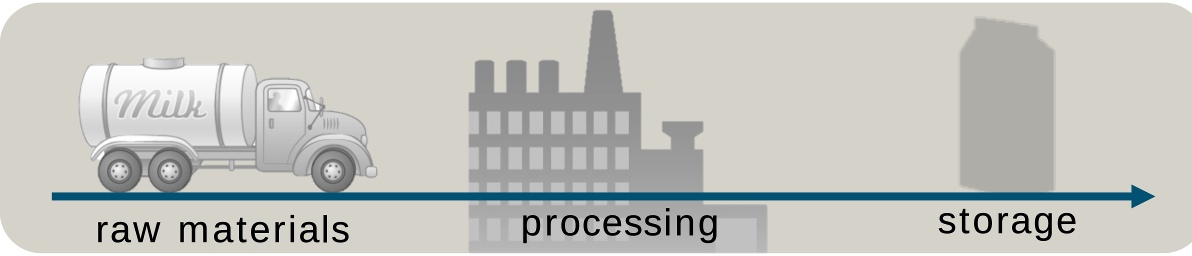
TI FOOD NUTRITION

What do we want to know?



- How will my troublemaker(s) behave?
- What are the sources and is the impact of variability?

What do we want to know?



sources

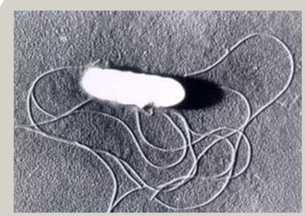
experimental

reproduction

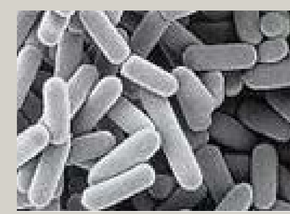
strain

history

- Why quantification of variability?
  - Rank importance
  - Realistic prediction



L. monocytogenes



L. plantarum

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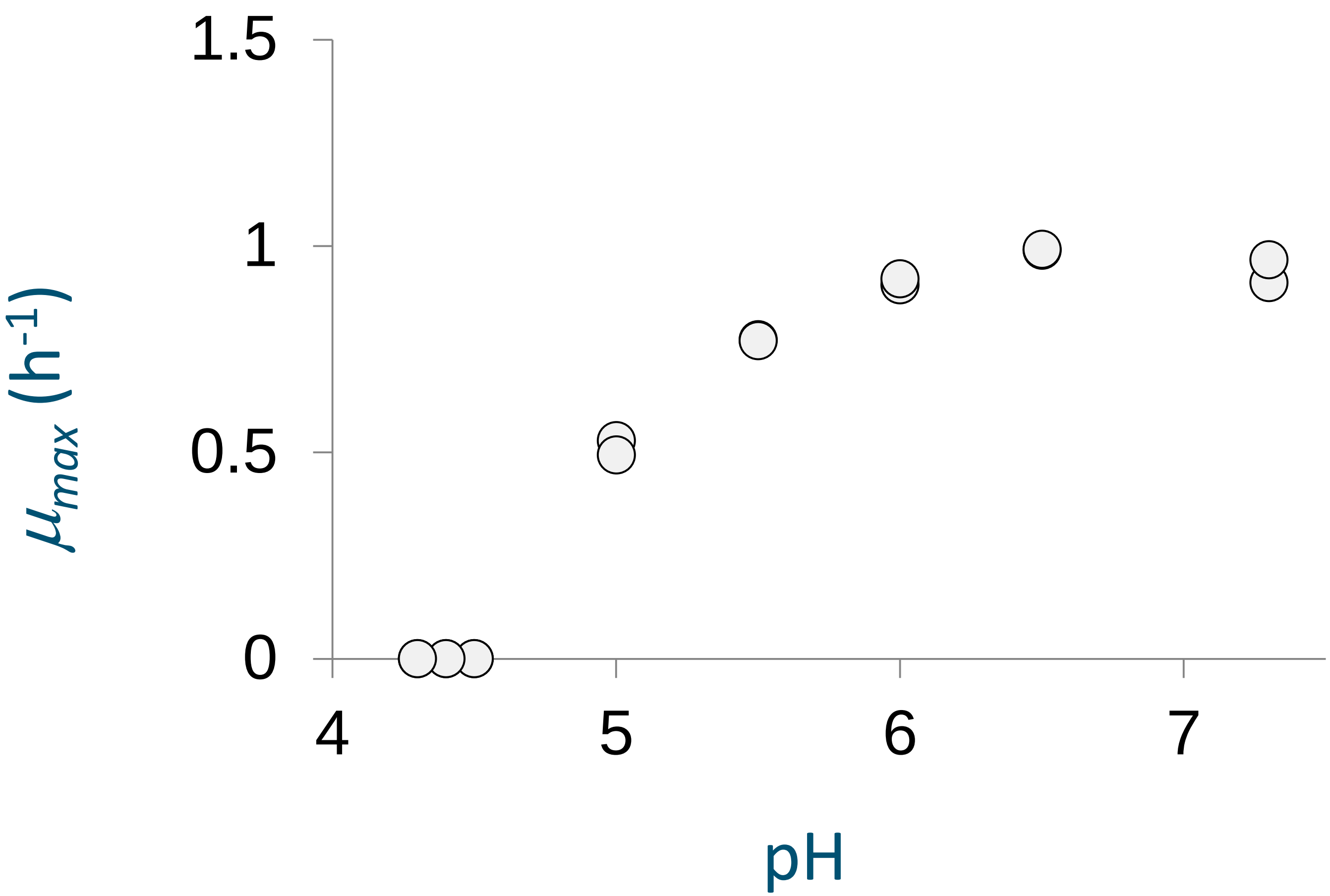
Variability in μ (pH) of *L. monocytogenes*



E

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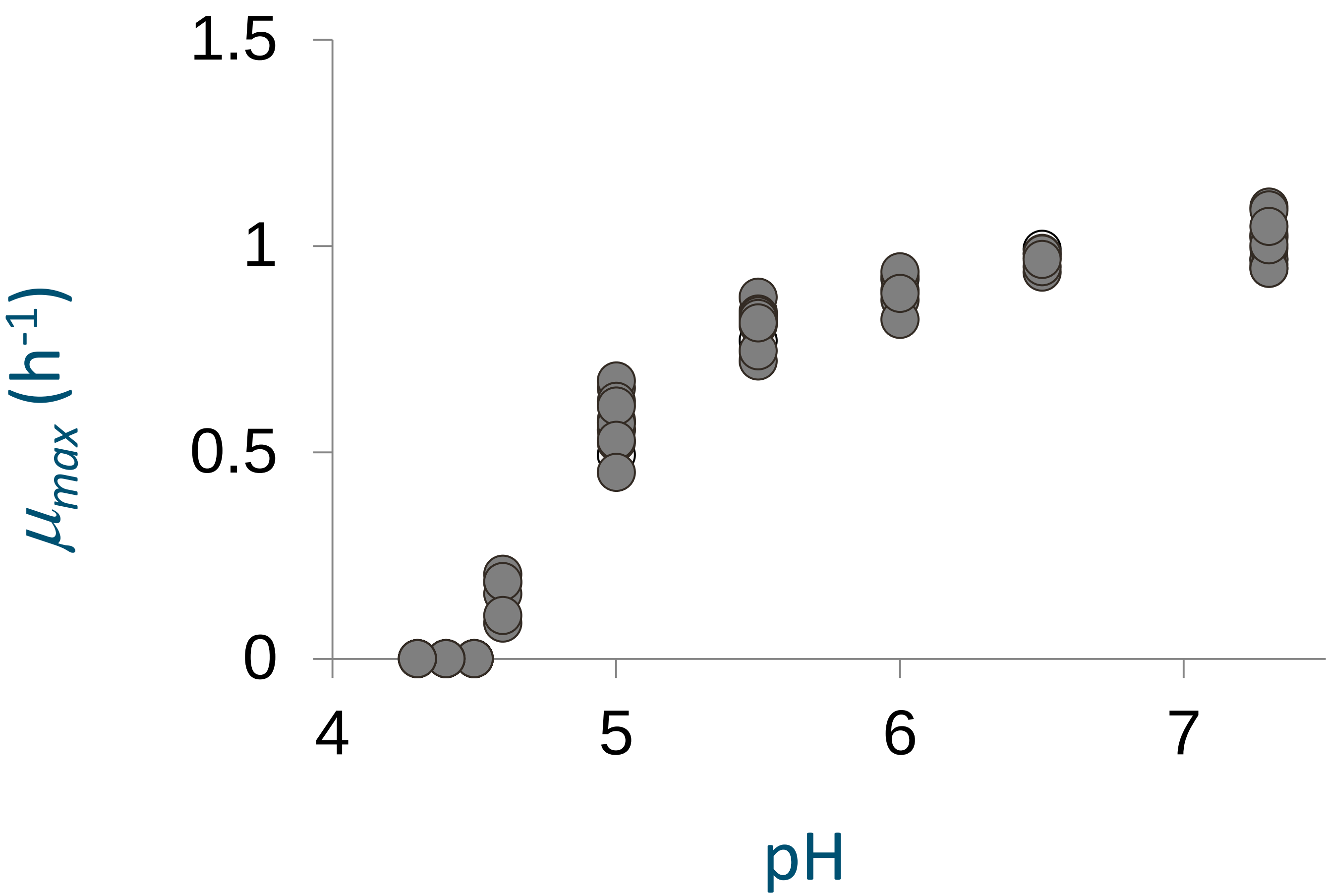
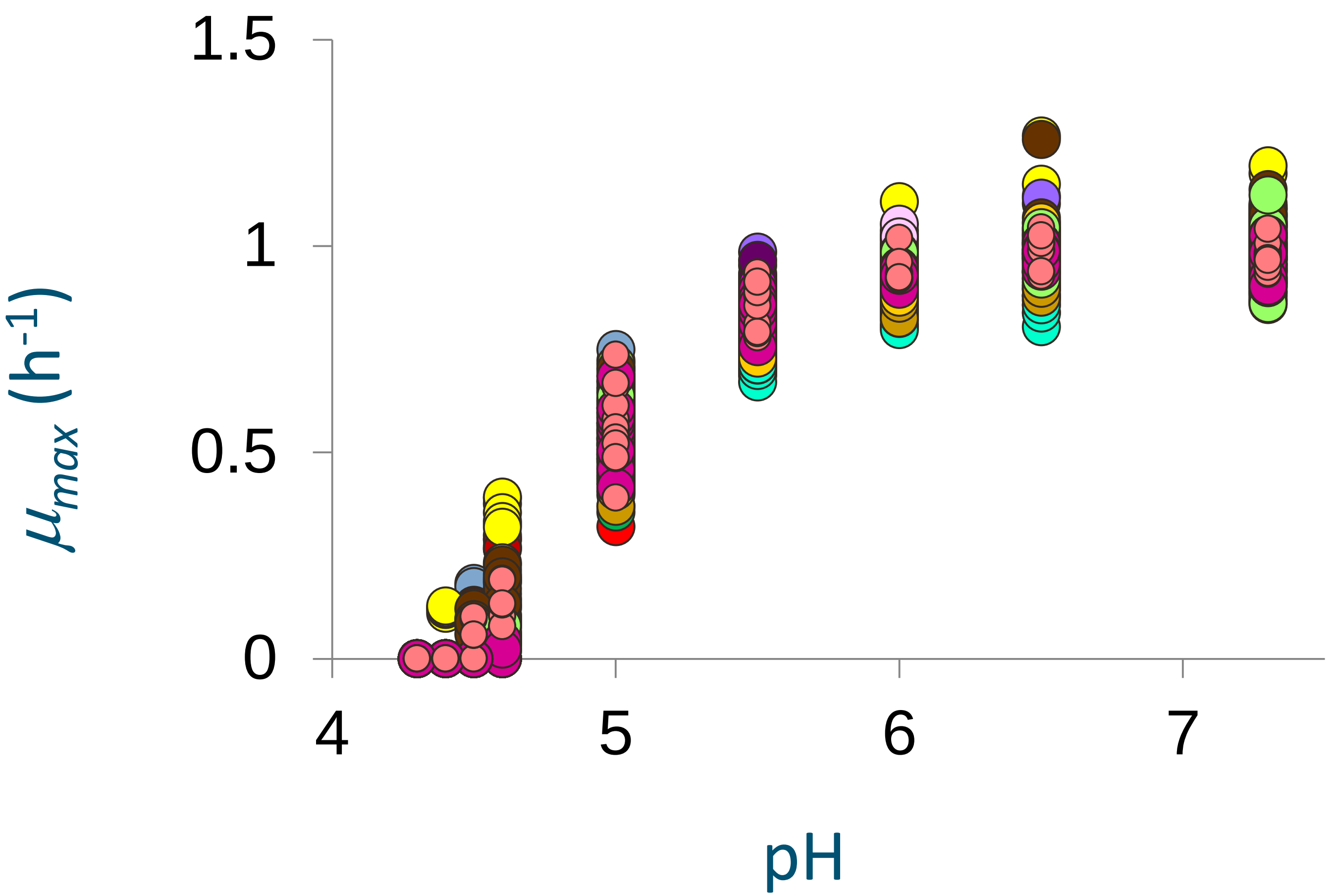
Variability in *μ* (pH) of *L. monocytogenes*



E

R

Variability in μ (pH) of *L. monocytogenes*



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R

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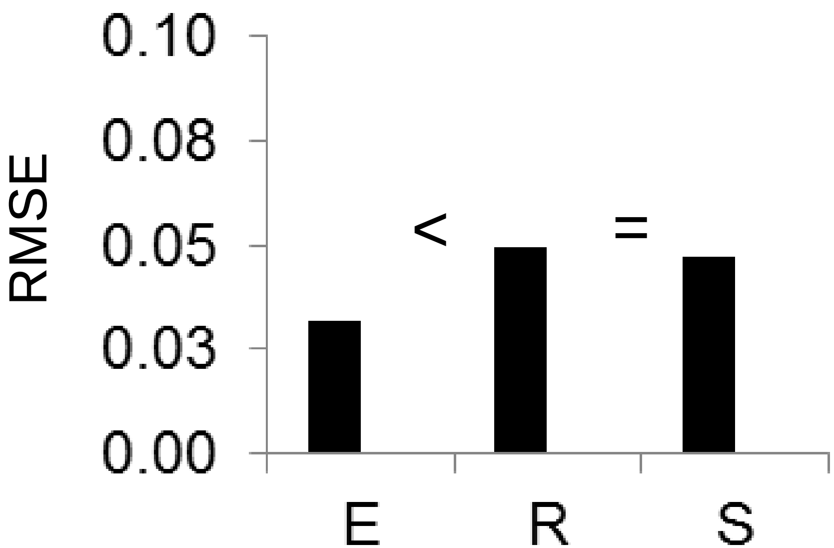
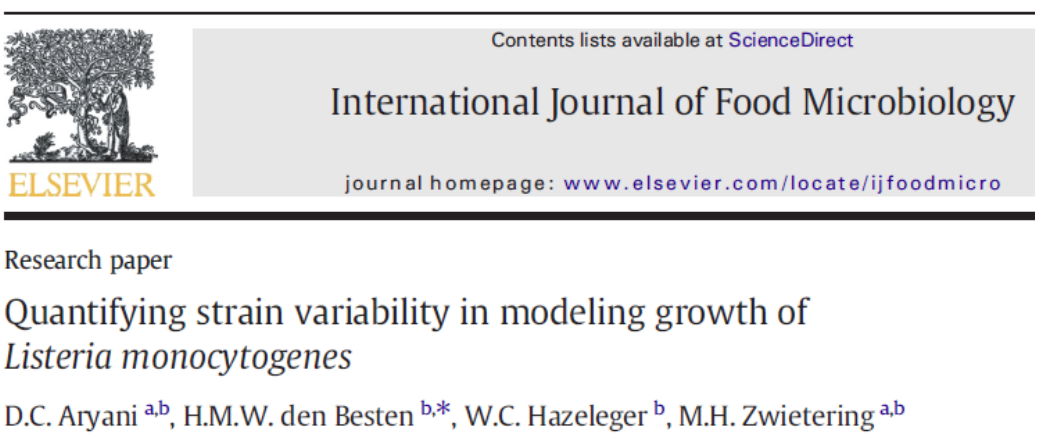
Variability in μ (pH) of *L. monocytogenes*



also for Temp and a_w



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International Journal of Food Microbiology

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

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]

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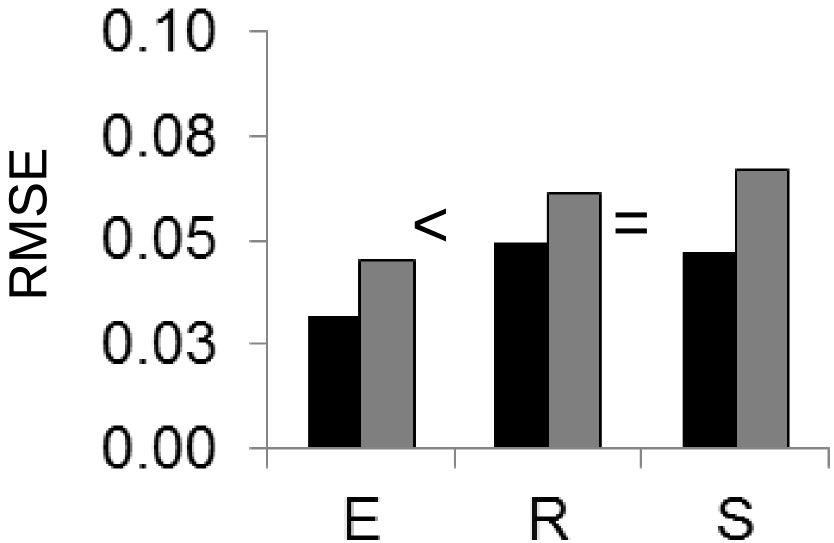
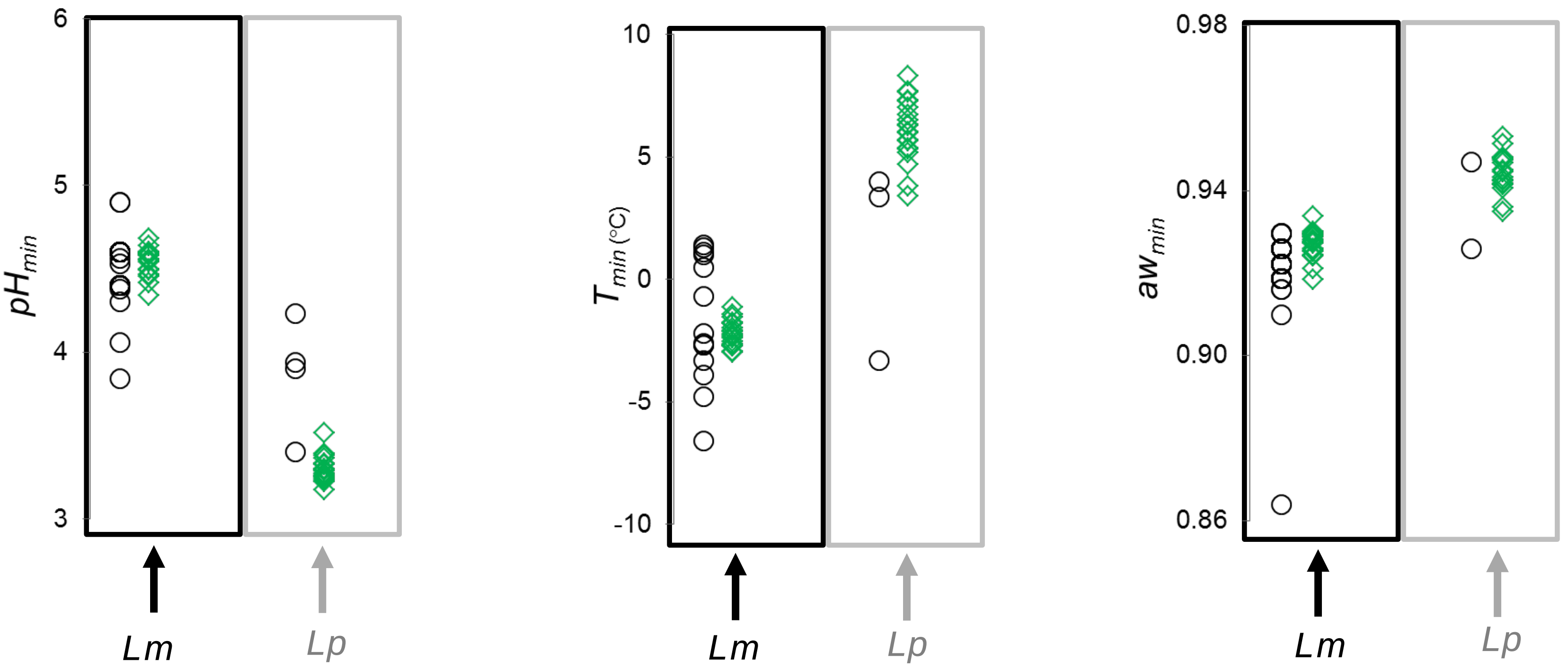
Variability in *μ* (pH) of *L. plantarum*



also for Temp and a_w

- Same trend for two distinct species

Variability in growth limits



- Strain variability explains ~ 50% of all variability as found in literature

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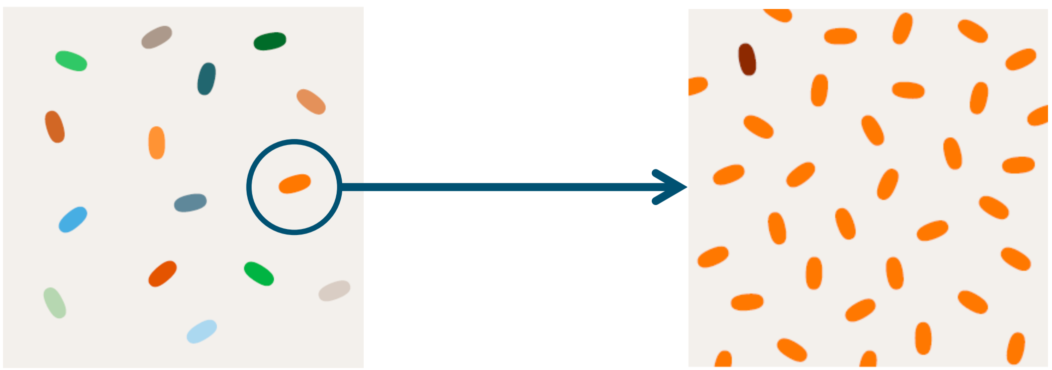
Impact of population heterogeneity



sources

experimental

reproduction

strain

single cells?

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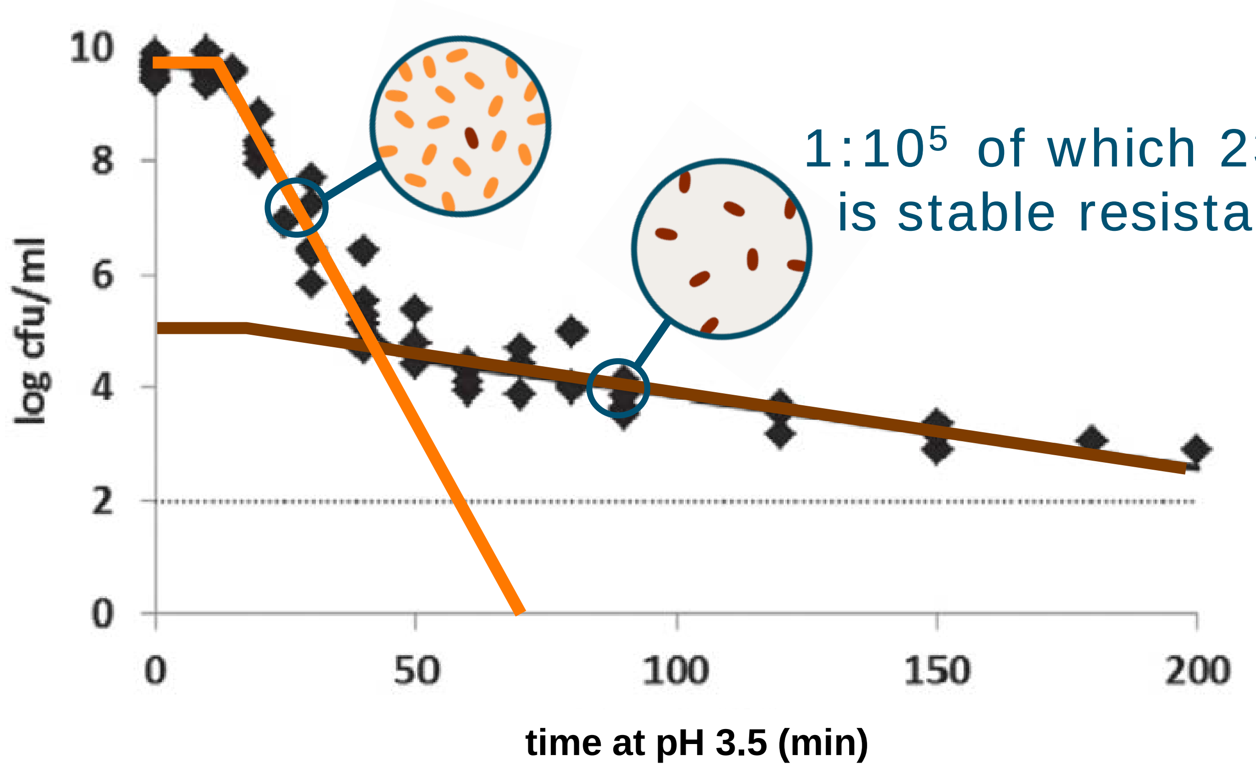
Impact of population heterogeneity



1:10^5 of which 23%
is stable resistant

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Isolation and quantification of highly acid resistant variants of *Listeria monocytogenes*

Karin I. Metselaar [a,b,c], Heidy M.W. den Besten [b], Tjakko Abee [a,b], Roy Moezelaar [a,c,1], Marcel H. Zwietering [a,b,*]

CrossMark

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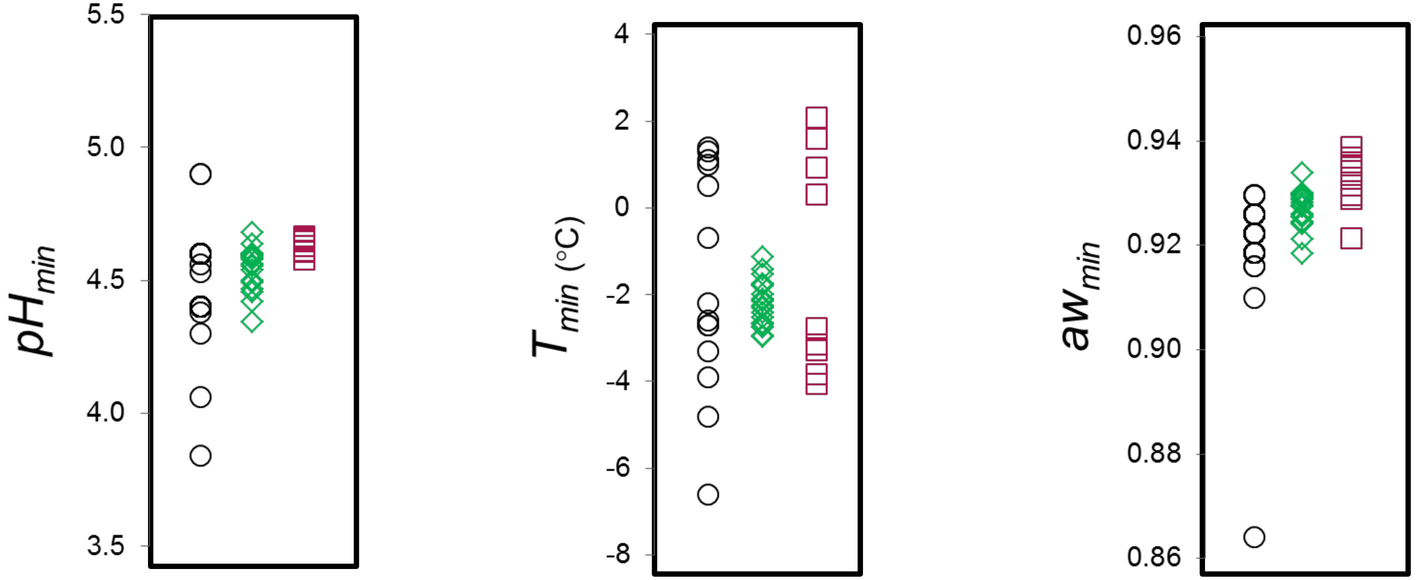
Characterisation of the variants

	Temperature	pH	Water activity	
	T_{min} (°C)	pH_{min}	$a_{w,\ min}$	μ_{opt} (h^{-1})
WT	-4.05	4.61	0.928	0.97
	[-7.48; -0.62]	[4.50; 4.71]	[0.921-0.935]	[0.89; 1.06]
3	-3.83	4.61	0.924	0.96
	[-6.76; -0.89]	[4.55; 4.67]	[0.916-0.932]	[0.87; 1.05]
7	-2.77	4.63	0.920	0.84
	[-4.28; -1.25]	[4.57; 4.69]	[0.910-0.930]	[0.75; 0.93]
9	2.08	4.58	0.921	0.49
	[0.26; 3.91]	[4.39; 4.77]	[0.894; 0.948]	[0.41; 0.57]
12	-3.29	4.63	0.922	0.65
	[-4.96; -1.61]	[4.56; 4.70]	[0.907-0.936]	[0.56; 0.74]
13	-3.15	4.66	0.937	0.70
	[-6.46; 0.15]	[4.61; 4.71]	[0.931-0.943]	[0.64; 0.77]
14	1.59	4.65	0.930	0.66
	[-0.30; 3.49]	[4.58; 4.72]	[0.920-0.940]	[0.57; 0.75]
15	0.31	4.66	0.933	0.67
	[-1.10; 1.72]	[4.59; 4.72]	[0.927-0.939]	[0.61; 0.73]

Metselaar et al., 2016, AEM

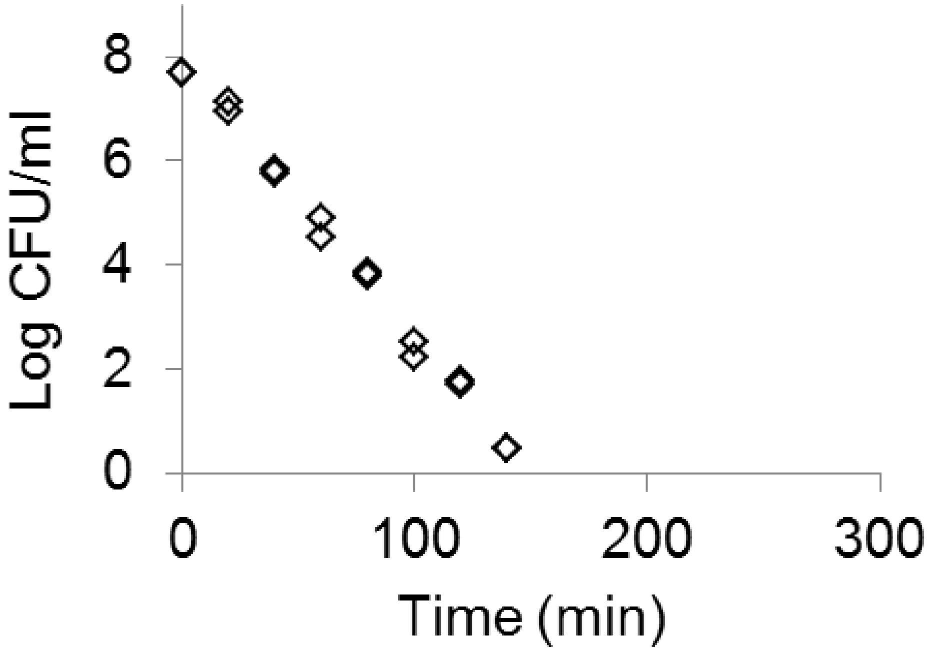
Variability in growth limits



- Variability within strain varies per stress
- Between and within strains variabilities explain 50-75% of all variability as found in literature

Variability in heat resistance

E



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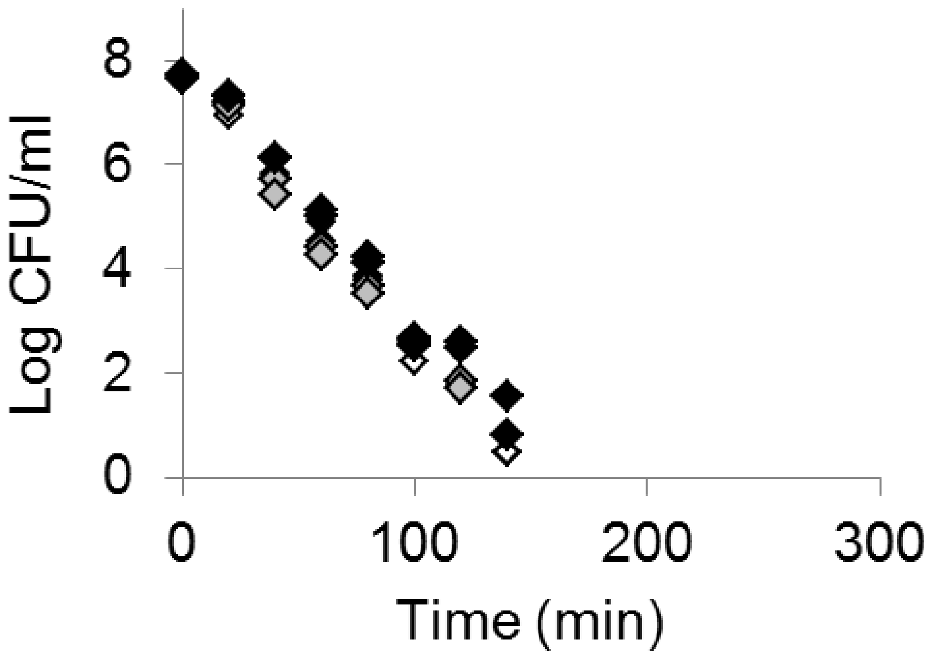
Quantifying variability on thermal resistance of *Listeria monocytogenes*

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

Variability in heat resistance

E

R



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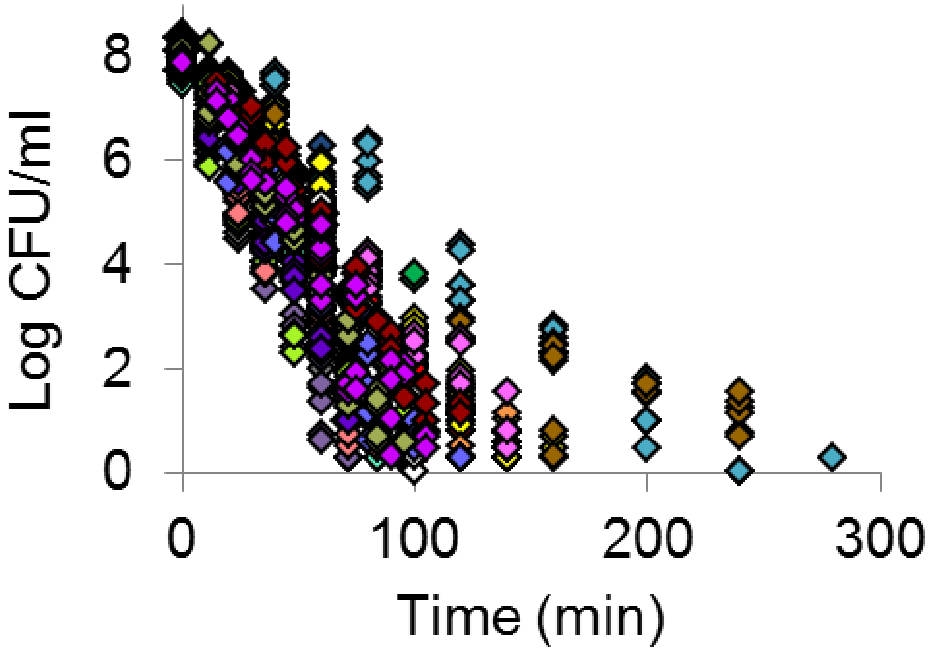
Variability in heat resistance



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R

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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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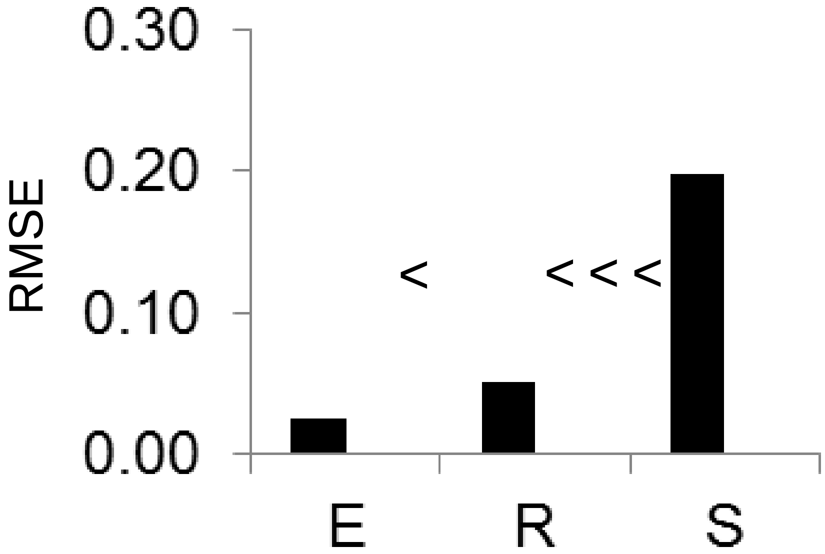
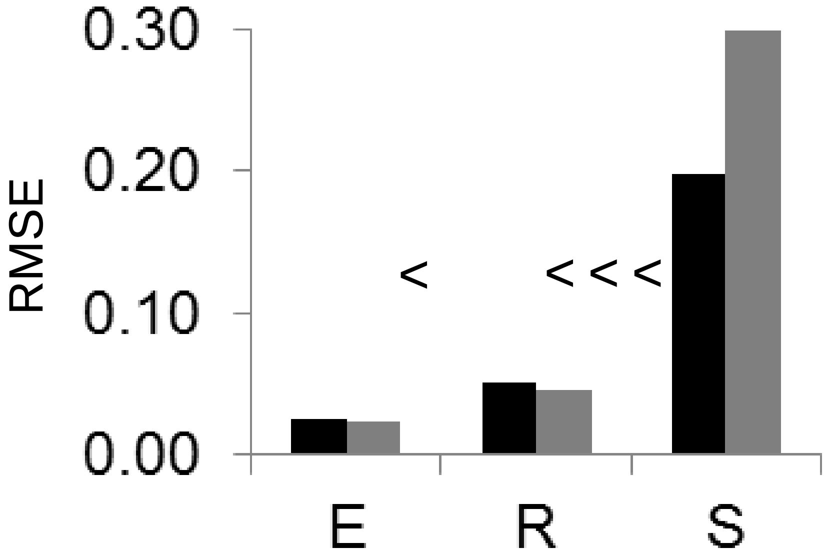
Quantifying variability in heat resistance



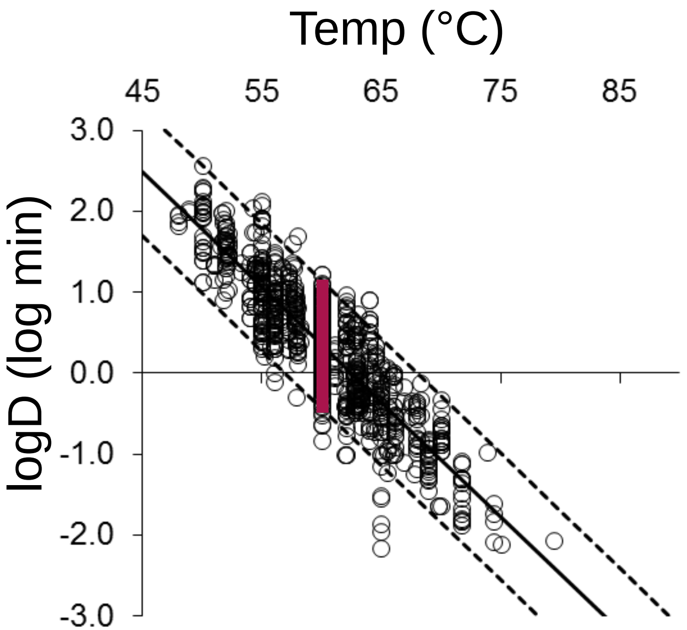
- Variability in *D*-value mainly determined by strain variability

Quantifying variability in heat resistance



- Variability in *D*-value mainly determined by strain variability for both species

Benchmarking heat resistance



Van Asselt & Zwietering, 2006

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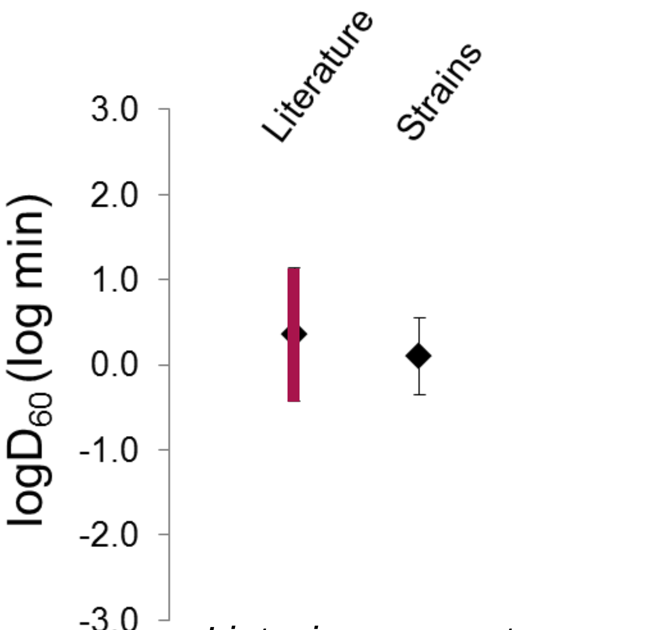
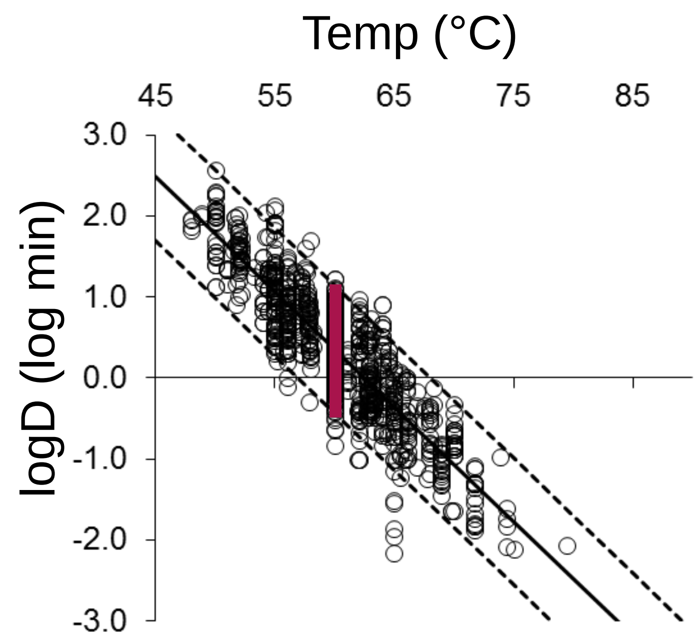
Benchmarking heat resistance



Listeria monocytogenes



Van Asselt & Zwietering, 2006

- Strain variability explains ~ 50% of variability as found in literature

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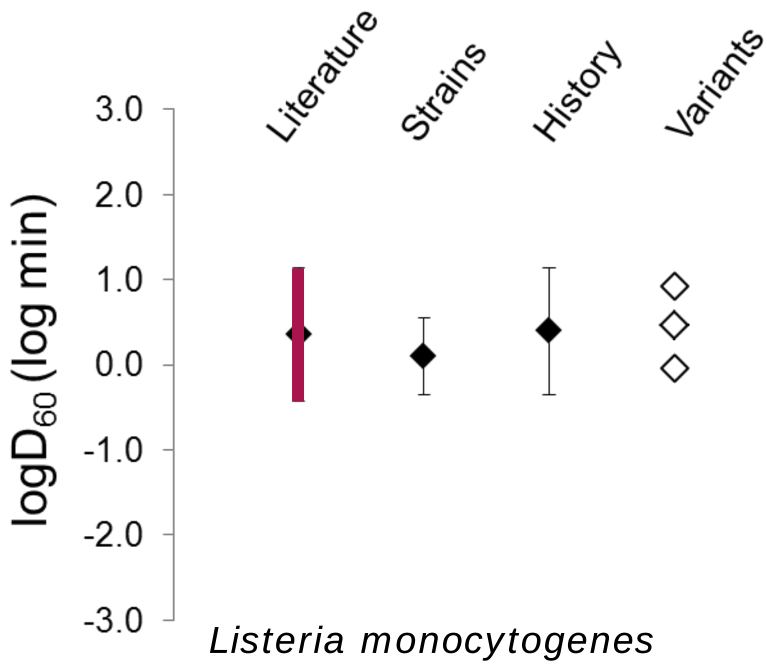
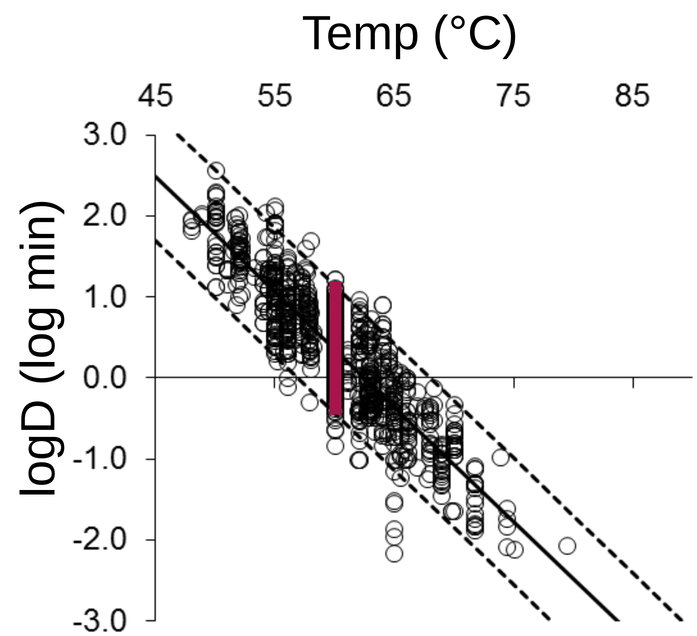
Benchmarking heat resistance

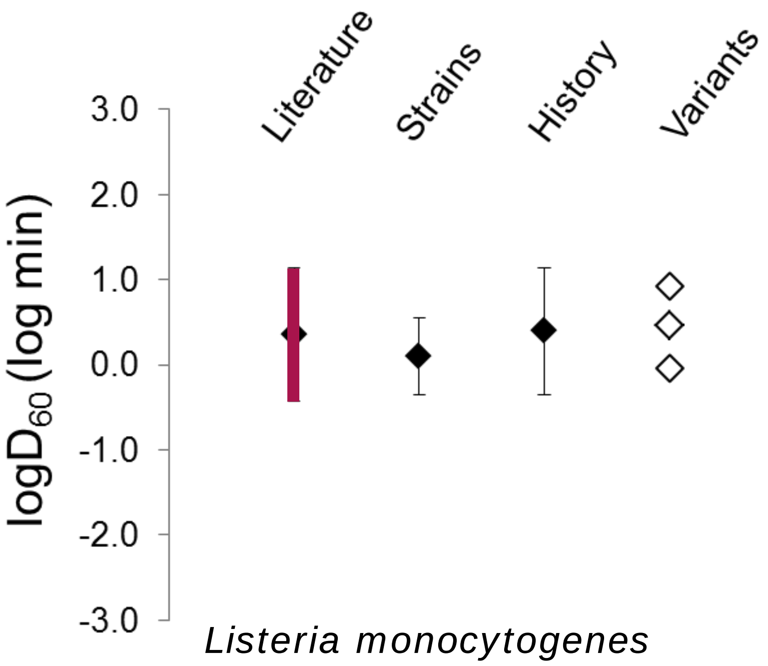


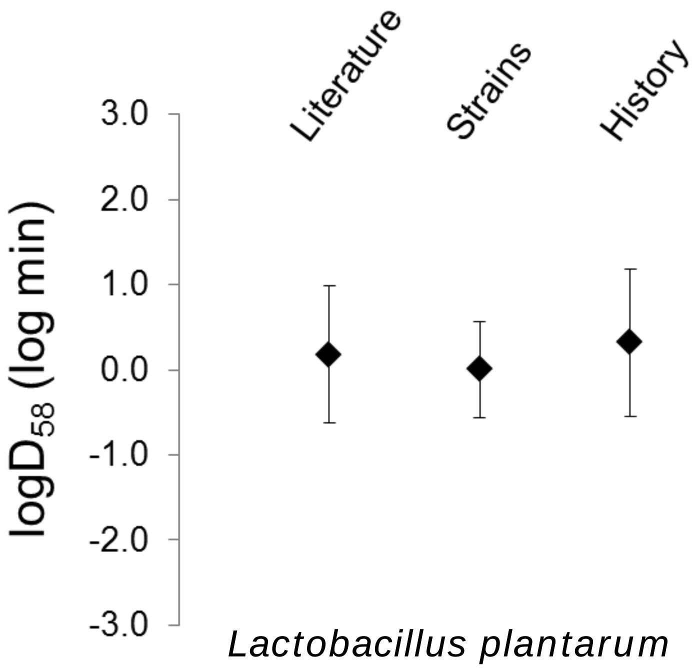


Van Asselt & Zwietering, 2006

- All variability as found in literature: fail–safe extremes
- Indeed, these extremes can be easily encountered

Benchmarking heat resistance





- Impact microbiological variability similar for both species

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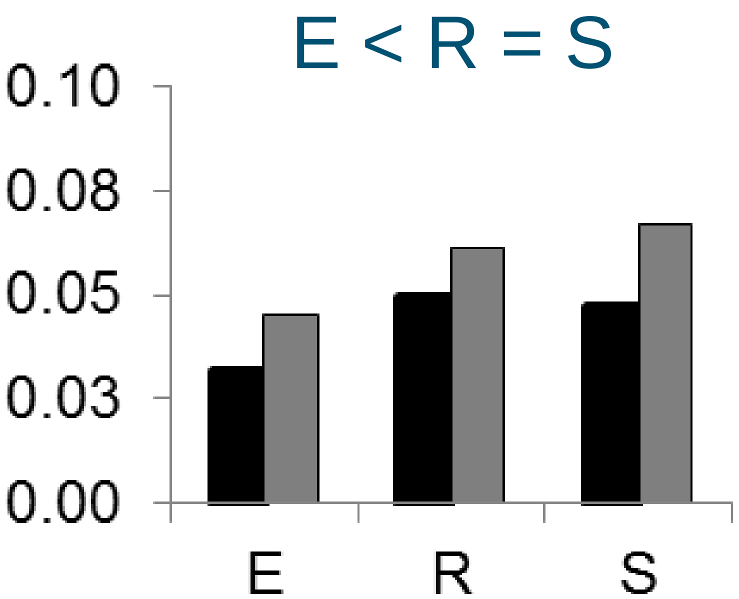
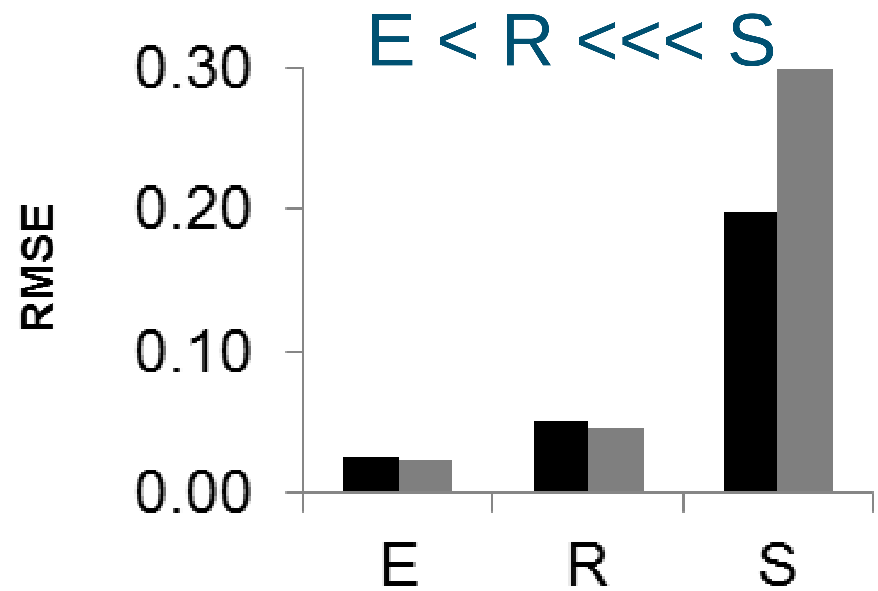
Prioritize sources of variability

μ(pH)



D-value



500-1000 points per variable

360 *D*-values

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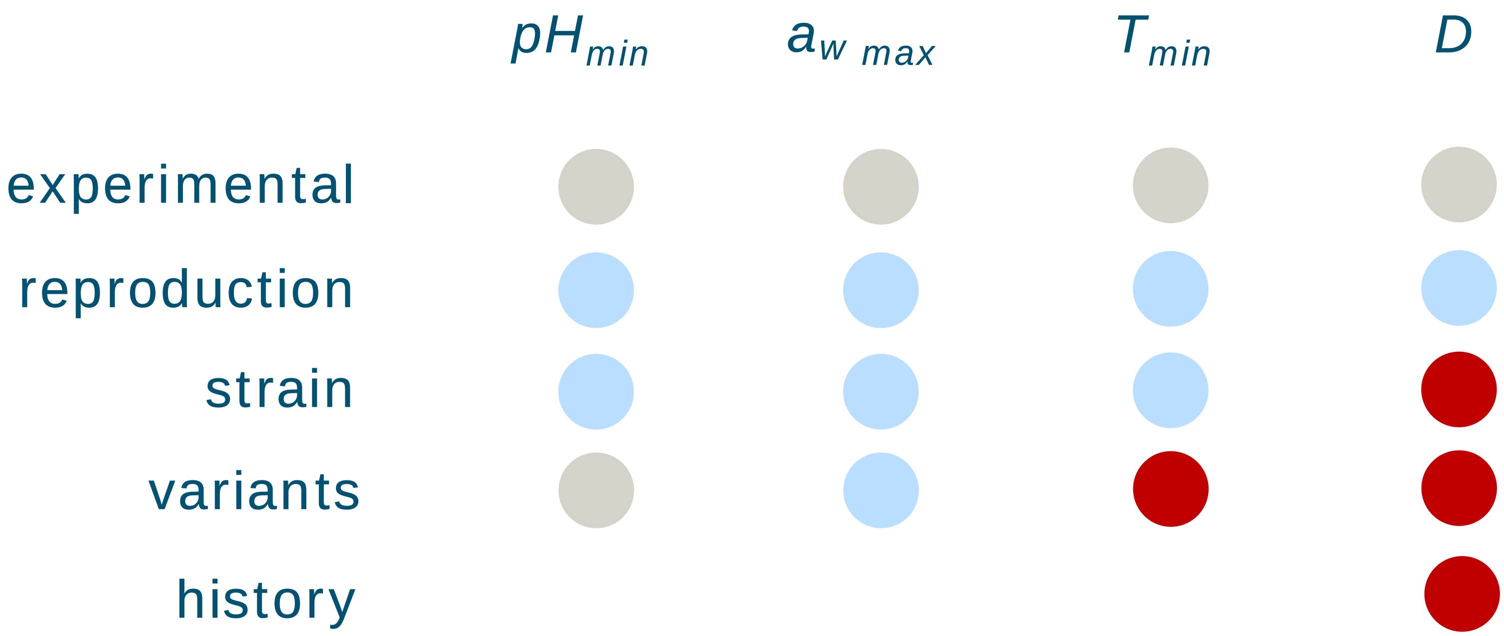
Prioritize sources of variability



- Sources of variability are quantified, then prioritize

Growth: E<R=S≤V

Heat resistance: E<R<<S=V=H

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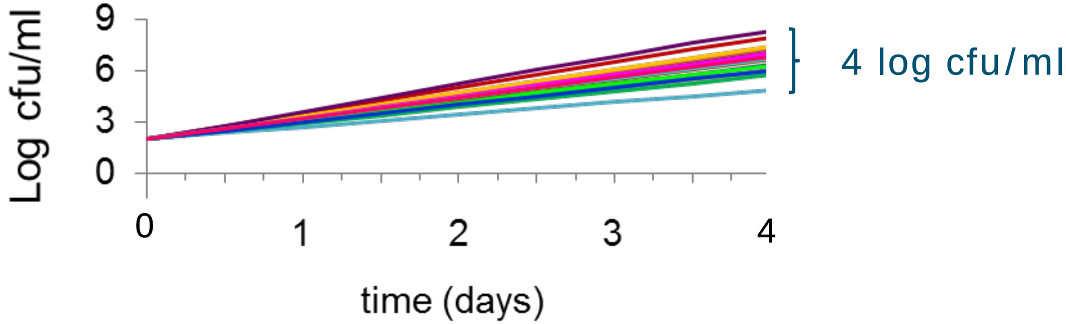
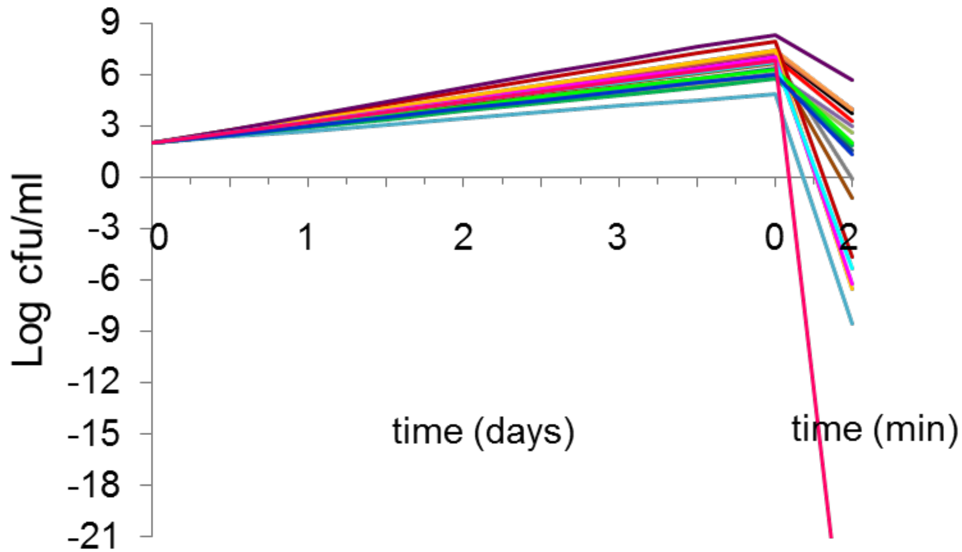
Impact of strain variability in growth and inactivation



Aryani et al., 2016

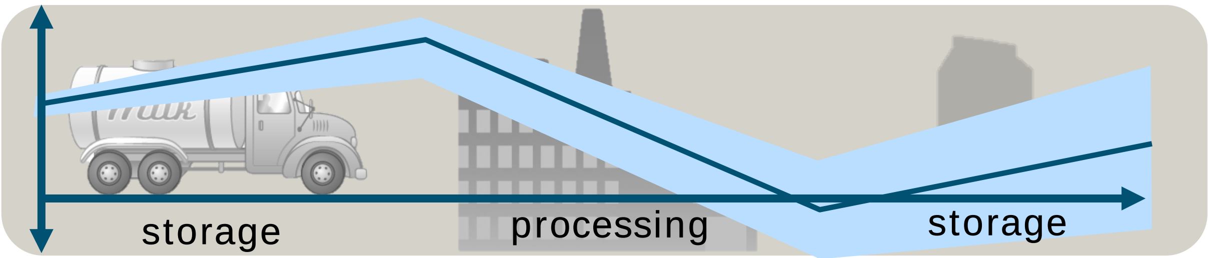
Impact of strain variability in growth and inactivation



- Impact of variability depends on your process

Aryani et al., 2016

Variability in the chain

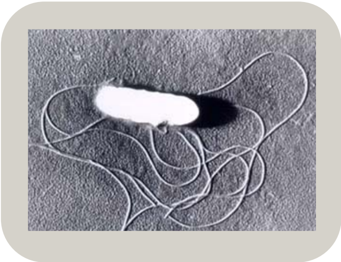


microbiological

initial contamination

storage T, t

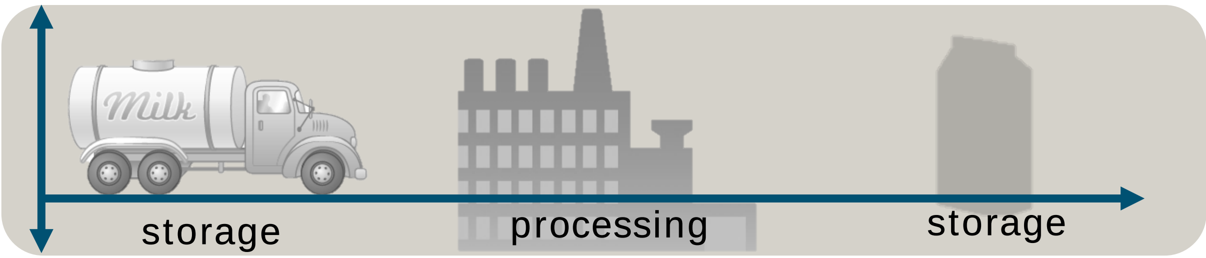
processing T, t



- More than only microbiological variability: comparison and ranking of impact

Scenario analysis

	storage	heating	storage	
	T=0-4°C t=2-72h	T=70°C σ=0.1°C t=15s, σ=0.1s	T=6.3°C, σ=2.7°C t=0-120h	Scenario 1
		T=70°C, σ=2°C		Scenario 2



- What when other chain factors are not well controlled?

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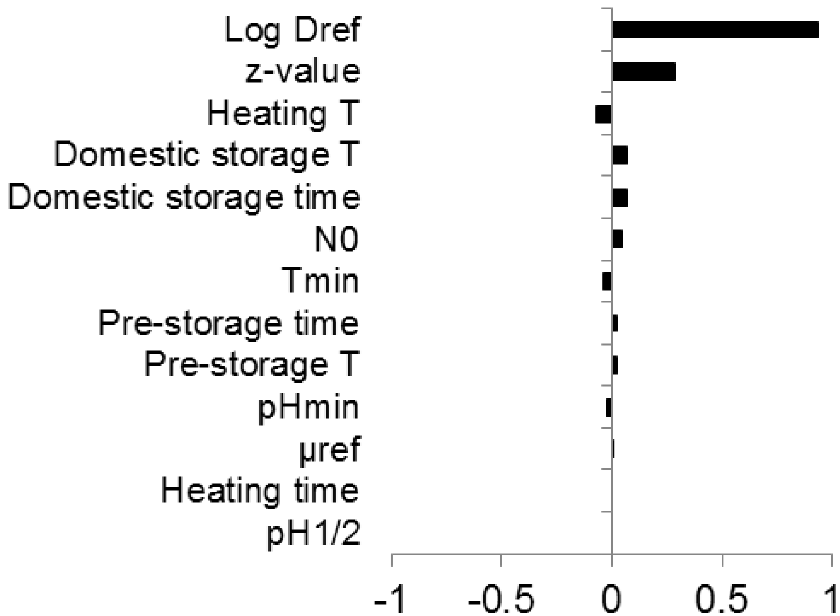
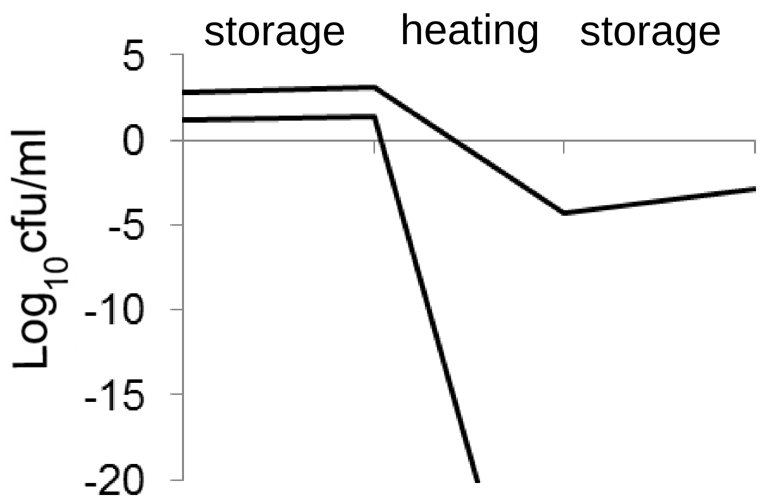
Scenario analysis

storage	heating	storage
T=0-4°C t=2-72h	T=70°C σ=0.1°C t=15s, σ=0.1s	T=6.3°C, σ=2.7°C t=0-120h

Scenario 1





- Impact of variability sources on variability in final contamination levels
- Huge impact of microbiological variability

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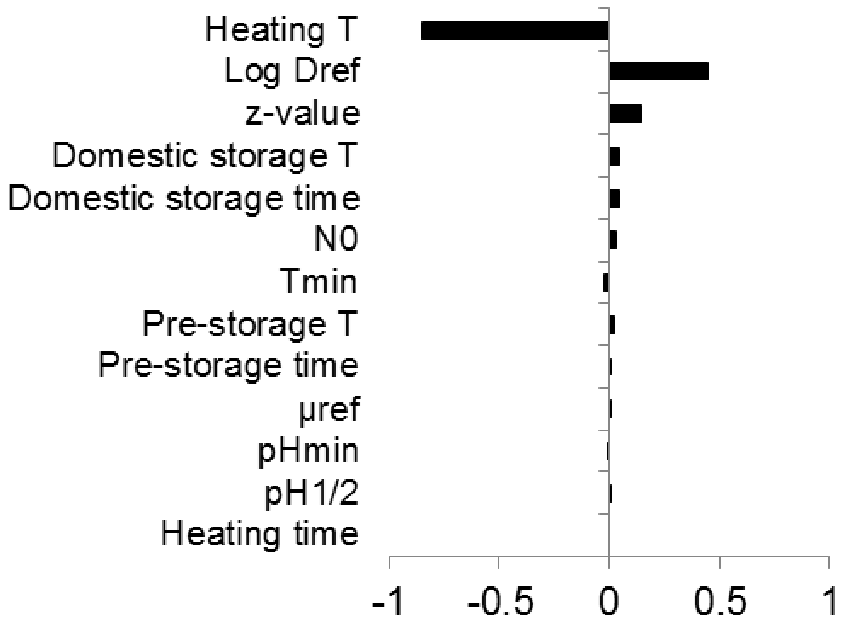
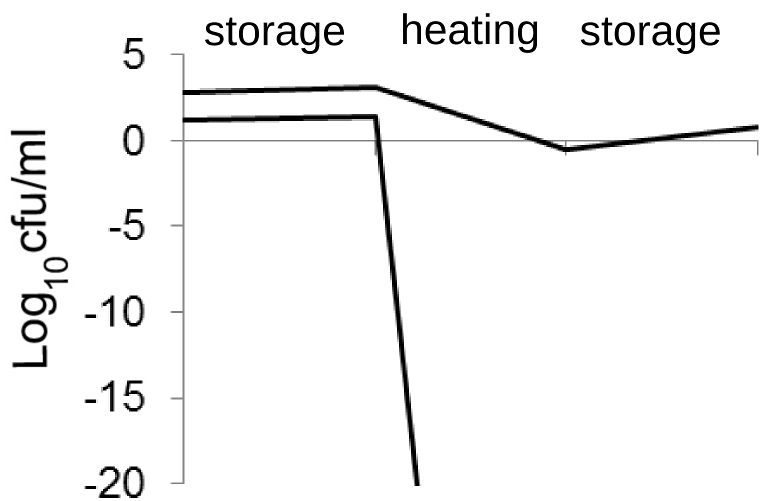
Scenario analysis

storage	heating	storage
T=0-4°C	T=70°C **σ=2°C**	T=6.3°C, σ=2.7°C
t=2-72h	t=15s, σ=0.1s	t=0-120h

Scenario 2





- Control where possible

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In conclusion

- Dive in variability sources but make link to practise: quantify, prioritize, benchmark
- What affects variability most?

  Strain variability, Growth history, Population heterogeneity, Process variability, ...
- Makes prediction realistic yet not more accurate

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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]

Co-production

Karin Metselaar

Diah Aryani

Marcel Zwietering

Tjakko Abee

L. monocytogenes strains

Strains	Origins	Serotype
ScottA	Human isolate from Massachusetts milk outbreak	4b
F2365	Jalisco cheese	4b
EGDe	Rabbit	1/2a
LO28	Healthy pregnant carrier	1/2c
AOPM3	Human isolate	4b
C5	Smoked meat	4b
H7764	Deli turkey	1/2a
H7962	Hotdog	4b
L6	Milk	1/2b
FBR12	Frozen vegetable mix	1/2a
FBR13	Frozen endive a la creme	1/2a
FBR14	Carrot piece	1/2a
FBR15	Ice cream packaging machine	1/2c
FBR16	Ham (after cutting machine)	1/2a
FBR17	Frozen fried rice	4d
FBR18	Ice cream	1/2a
FBR19	Frozen meat	1/2a
FBR20	Frozen vegetables for soup	1/2a
FBR21	Fresh yeast	4d
FBR33	Pancake	1/2c

L. plantarum strains

Strains	Isolation source
FBR01	Dressing
FBR02	Dressing
FBR03	Salad dressing
FBR04	Cheese with garlic
FBR05	Dressing
FBR06	Onion ketchup
FBR22	Sausage
FBR23	Potato salad
FBR24	Luncheon meat
FBR25	Sliced salami
FBR26	Frankfurter
FBR27	Sliced cooked ham
FBR28	Spoiled tomato ketchup
FBR29	Lettuce
FBR30	Raw vegetable salad
WCFS1	Human saliva
LMG18035	Milk
LMG23454	Healthy adult faeces
LMG6907	Pickled cabbage
SF2A35B	Sour cassava

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