

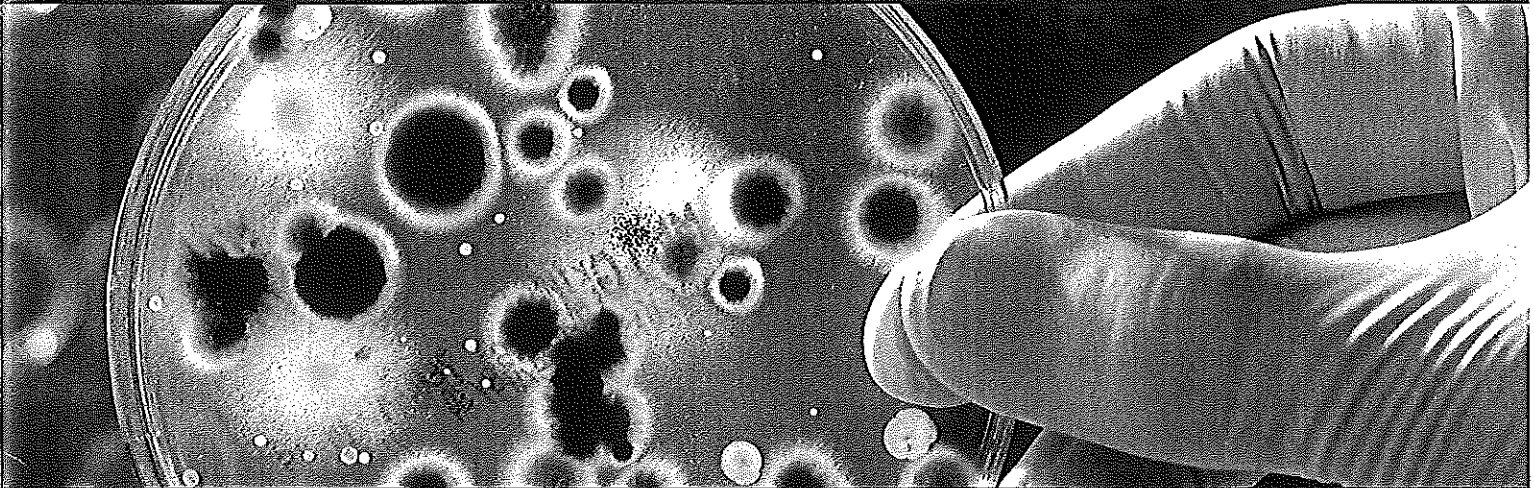


14th Australian Food Microbiology Conference
2nd IAFP Asia Pacific Symposium on Food Safety

Securing Global Food Safety

14th Australian Food Microbiology Conference
2nd IAFP Asia Pacific Symposium on Food Safety

Meet the world's best in Australia



The inaugural meeting of AIFST, ICMSF, IAFP
26-28 September 2011
Sebel Albert Park, Melbourne, Australia

PROGRAM & REGISTRATION

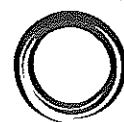
www.aifst.com.au/foodsafety



the Australian Institute of
food science and technology
incorporated



International Commission on
Microbiological Specifications
for Foods (ICMSF)



International Association for
Food Protection

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Linking genomics and physiology in quantitative risk assessment

MARCEL H. ZWIETERING

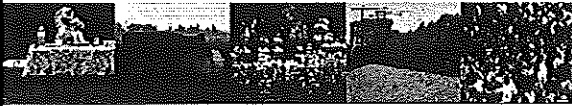
Laboratory of Food Microbiology, Wageningen University, Wageningen, The Netherlands

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Two important developments of the last decades are the large progress made in the domain of genomics and in the development and use of quantitative microbiological risk assessment (QMRA) within food safety management. Clearly omics technologies provide very relevant information for detection and typing of organisms and in investigating the ecology within food products and their environment. Furthermore omics is very productive in getting mechanistic insight in organism behavior in the food chain, both for their reaction to process or product conditions as well as for their virulence behavior. On the other hand QMRAs are developed and used to investigate quantitatively risks of pathogens, the effects of interventions and to set process and product criteria. The large amount of specific data supplied by genomics tools can not directly and easily be incorporated in QMRA, since there is a big gap between the input parameters of QMRAs and the results from genomics experiments. The insight obtained by genomics research, the mechanisms found, and certain specific results can be certainly efficiently used to improve QMRAs. An iterative approach going from chain and quantitative level to molecular level and back might be a way forward to better link the fast amount of genomics results and QMRAs. Going from molecular level to management, every time data needs to be generalized and lumped, within a food safety systems biology approach.

Linking genomics and physiology in quantitative risk assessment

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Laboratory of Food Microbiology
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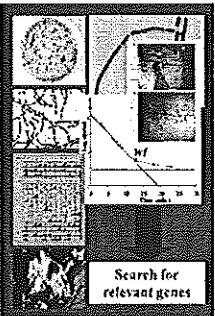
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From molecule to management

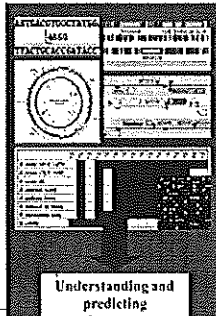
- Genomics
 - Sequence(s): ultimate identification, specific traits, BIO-IT
 - DNA-arrays: identification, specific traits
 - PCR: specific traits, identification
 - AFLP, DGGE: identification
- Transcriptomics
 - micro-arrays: RNA-levels under specific conditions
- Proteomics
 - Gel Electrophoresis, MALDITOF: protein levels
- Metabolomics
 - phenotypic microarray, HPLC, NMR, etc.

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phenotype to genotype genotype to phenotype

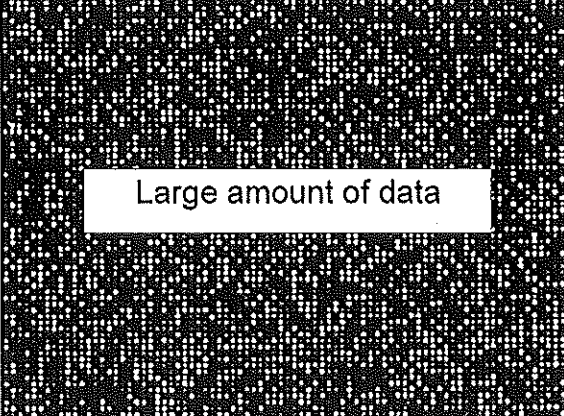


Search for relevant genes



Understanding and predicting phenotypes

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Large amount of data

From molecule to management

- sequence
- annotation

Accession	Accession	Accession	Accession	Accession	Accession	Accession	Accession	Accession	Accession
U00096	U00097	U00098	U00099	U00100	U00101	U00102	U00103	U00104	U00105
1	2	3	4	5	6	7	8	9	10

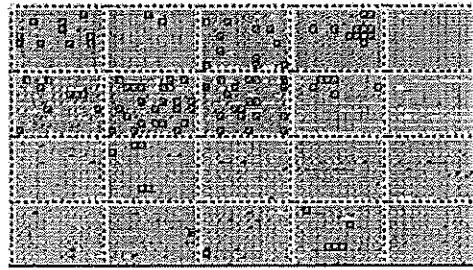
- effect ? active ? (point mutations, gene-regulation)

Metabolic capacity of *Bacillus cereus* strains
ATCC 14579 and ATCC 10987 interlinked with
comparative genomics

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From molecule to management

phenotypic microarray



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From molecule to management

understanding of mechanisms

in silico characteristics needs to be confirmed biochemically or phenotypically

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Ecology

Application of pulsed field gel electrophoresis to characterize and trace the prevalence of *Listeria* in soft-ripened cheese and cheese factories

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QRAR/Qual

Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods

Center for Food Safety and Applied Nutrition
Food and Drug Administration
U.S. Department of Health and Human Services

Food Safety and Inspection Service
U.S. Department of Agriculture

September 2003

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QMRA

- N_0
- $\mu(t) + T + t + N_{max}$
- N_t (cooking)
- serving size
- Dose
- $P_{ill} = 1 - \exp(-r \cdot D) + \text{mortality}$

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QMRA

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Kinetics

Multiple products / variability

Variability distribution of μ_{ref}

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QMRA

Prevalence and Concentration

Temperature
Time
Serving Size

EDR
MDP

N
D

Uncertainty
Enteric risk

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Getting insight with functional genomics, phenotype and mathematics

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Non-linear inactivation described in HHP

$$\log \left(\frac{N_t}{N_0} \right) = \log \left((1-f) + 10 \frac{t}{D_{90}} + f + 10 \frac{t}{D_{99}} \right)$$

Survival (log CFU/ml)

Time (min)

EGDg
L028
Scott A

Inactivation Kinetics of Three *Listeria* monocytogenes Strains under High Hydrostatic Pressure

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Investigate mechanism + quantitative modelling

$$\log \left(\frac{N_t}{N_0} \right) = \log \left((1-f) + 10 \frac{t}{D_{90}} + f + 10 \frac{t}{D_{99}} \right)$$

Survival (log CFU/ml)

Time (min)

Stable survivors
only for L028 and Scott A

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Robustness

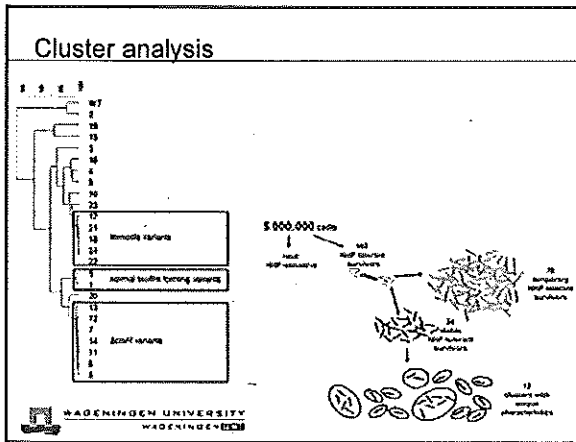
- HHP (350 MPa, 20 min)
- Heat (60 C, 1 min)
- pH (2.5, 3 min)

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Large dataset

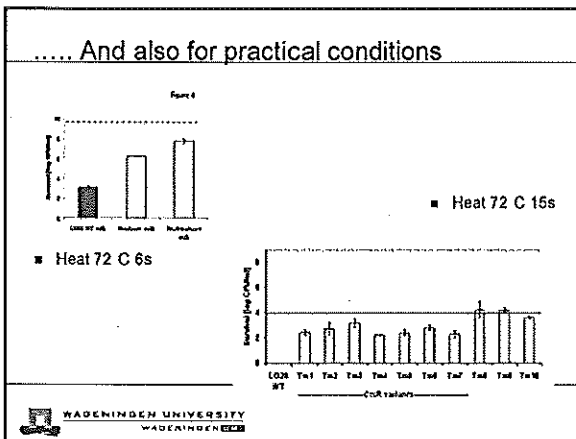
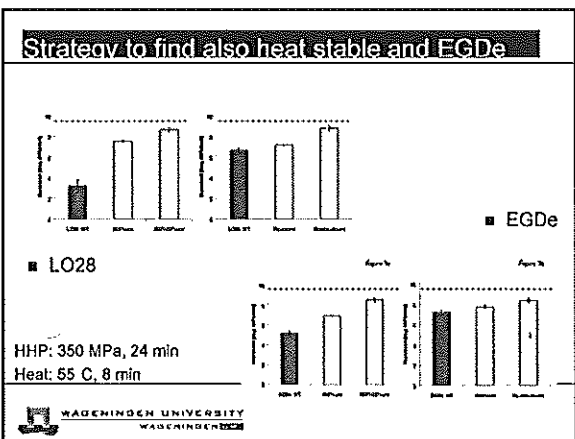
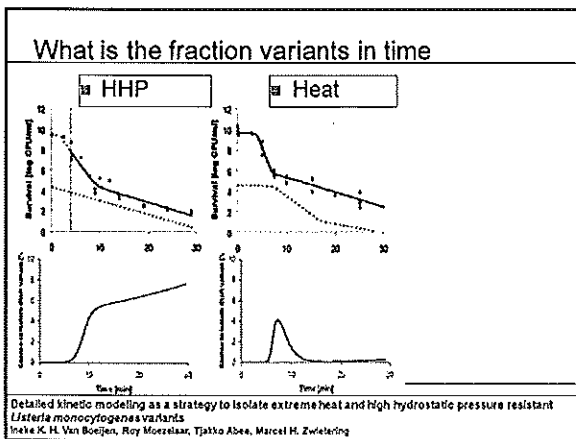
Strain	Genotype	Survival (log CFU/ml)	Time (min)	Pressure (MPa)	Temp (C)	pH	Other parameters
1	EGDg	1.00	0	350	60	2.5	...
2	EGDg	1.00	15	350	60	2.5	...
3	EGDg	1.00	30	350	60	2.5	...
4	EGDg	1.00	45	350	60	2.5	...
5	EGDg	1.00	60	350	60	2.5	...
6	EGDg	1.00	75	350	60	2.5	...
7	EGDg	1.00	90	350	60	2.5	...
8	EGDg	1.00	105	350	60	2.5	...
9	EGDg	1.00	120	350	60	2.5	...
10	EGDg	1.00	135	350	60	2.5	...
11	EGDg	1.00	150	350	60	2.5	...
12	EGDg	1.00	165	350	60	2.5	...
13	EGDg	1.00	180	350	60	2.5	...
14	EGDg	1.00	195	350	60	2.5	...
15	EGDg	1.00	210	350	60	2.5	...
16	EGDg	1.00	225	350	60	2.5	...
17	EGDg	1.00	240	350	60	2.5	...
18	EGDg	1.00	255	350	60	2.5	...
19	EGDg	1.00	270	350	60	2.5	...
20	EGDg	1.00	285	350	60	2.5	...
21	EGDg	1.00	300	350	60	2.5	...
22	EGDg	1.00	315	350	60	2.5	...
23	EGDg	1.00	330	350	60	2.5	...
24	EGDg	1.00	345	350	60	2.5	...
25	EGDg	1.00	360	350	60	2.5	...
26	EGDg	1.00	375	350	60	2.5	...
27	EGDg	1.00	390	350	60	2.5	...
28	EGDg	1.00	405	350	60	2.5	...
29	EGDg	1.00	420	350	60	2.5	...
30	EGDg	1.00	435	350	60	2.5	...
31	EGDg	1.00	450	350	60	2.5	...
32	EGDg	1.00	465	350	60	2.5	...
33	EGDg	1.00	480	350	60	2.5	...
34	EGDg	1.00	495	350	60	2.5	...
35	EGDg	1.00	510	350	60	2.5	...
36	EGDg	1.00	525	350	60	2.5	...
37	EGDg	1.00	540	350	60	2.5	...
38	EGDg	1.00	555	350	60	2.5	...
39	EGDg	1.00	570	350	60	2.5	...
40	EGDg	1.00	585	350	60	2.5	...
41	EGDg	1.00	600	350	60	2.5	...
42	EGDg	1.00	615	350	60	2.5	...
43	EGDg	1.00	630	350	60	2.5	...
44	EGDg	1.00	645	350	60	2.5	...
45	EGDg	1.00	660	350	60	2.5	...
46	EGDg	1.00	675	350	60	2.5	...
47	EGDg	1.00	690	350	60	2.5	...
48	EGDg	1.00	705	350	60	2.5	...
49	EGDg	1.00	720	350	60	2.5	...
50	EGDg	1.00	735	350	60	2.5	...

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Specific finding or more widespread ?

- All three strains non-linear inactivation
- No stable variants found for EGDe
- No stable variants found for heat inactivation



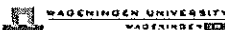
Strength of modelling

- Widespread presence of stable resistant variants for all three strains and both for HHP and heat
- Most have robust characteristics
- Strength of kinetic modelling in
 - unravelling the causes of non-linear inactivation
 - optimising experimental procedures for hypothesis testing
 - strategy to find the needle in the haystack

Genomics

sequencing to determine characteristics and mechanism of the non CtsR mutants

If target sequences known, q-PCR can be used to determine fraction resistant variants

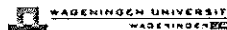


Generalising / lumping / selective integration
omics data (sequence, transcriptomes,)

Insight, prevalences and fractions

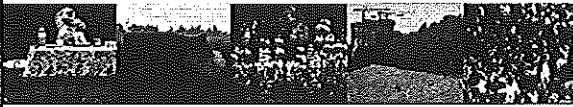
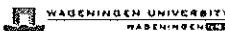
quantitative models

QMRA



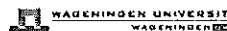
Thank you

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Overall analysis

Exposure Assessment	Dose-response	Risk characterisation	Risk management
C			
No	Nt	p(Nt)	np
GR (D/z, μ, λ)			P
			Tolerable ?
	FSO		ALOP



No

C

$\Sigma G \Sigma R$
(D/z, μ, λ)

Nt

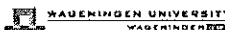
p(D)

np

P


Process Variability
multiple phenomena

Medium representativity
strain variability
preculture representativity
repetition error
experimental error (Petri dish)



Non-linear inactivation: Stress resistant variants ?

- Non-linear inactivation kinetics: kinetic modelling
- Strains (Scott A, EGDe, LO28)
- All due to stable variants ?
- Characterisation variants
- Only for HHP or also in heat inactivation ?



No stable variants for EGDe

Strain	f_{EGDe}	Stable f_{EGDe}	
EGDe	8.1×10^{-6}		0% (0 out of 102)
LO28	2.1×10^{-5}	4.9×10^{-6}	24% (24 out of 102)
Scott A ²	3.0×10^{-5}	1.2×10^{-5}	40% (33 out of 84)

*calculated from Karatzas, Valdramidis and Bennik (2005)

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Characterisation stable LO28 variants (24)

- Robustness and growth performance in range of food-relevant conditions
- Characteristics relevant for infection
- Genetic analysis

Population Diversity of *Listeria monocytogenes* LO28, Phenotypic and Genotypic Characterisation of Variants Resistant to High Hydrostatic Pressure

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