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

Securing Global Food Safety

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2nd IAFP Asia Pacific Symposium on Food Safety

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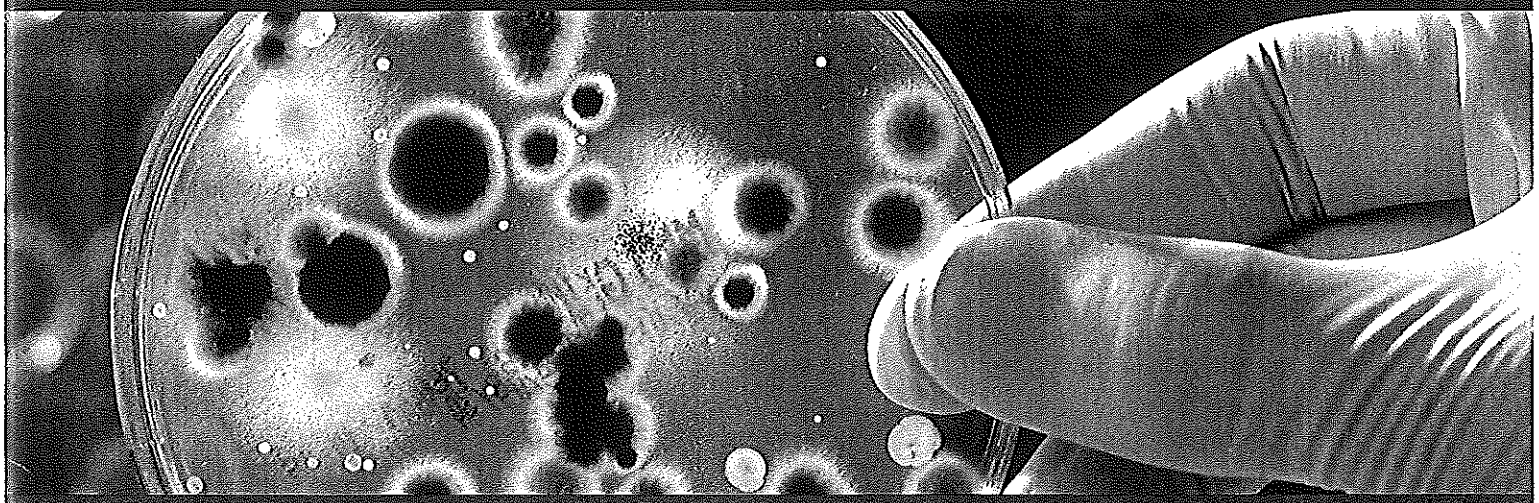

The inaugural meeting of AIFST, ICMSF, IAFP

26-28 September 2011

Sebel Albert Park, Melbourne, Australia

PROGRAM & REGISTRATION

www.aifst.com.au/foodsafety

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International Commission on Microbiological Specifications for Foods (ICMSF)

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Linking genomics and physiology in quantitative risk assessment

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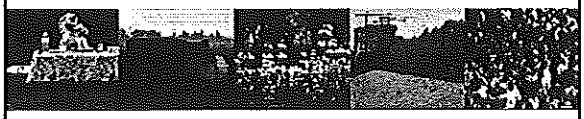
Two important developments of the last decades are the large progress made in the domain of genomics and in the development and use of quantitative microbiological risk assessment (QMRA) within food safety management. Clearly omics technologies provide very relevant information for detection and typing 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

Marcel Zwietering
Laboratory of Food Microbiology
Wageningen University
The Netherlands



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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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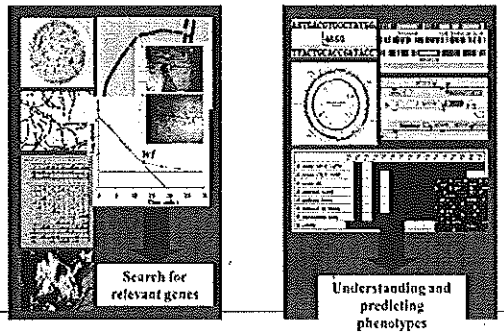
phenotype to genotype genotype to phenotype



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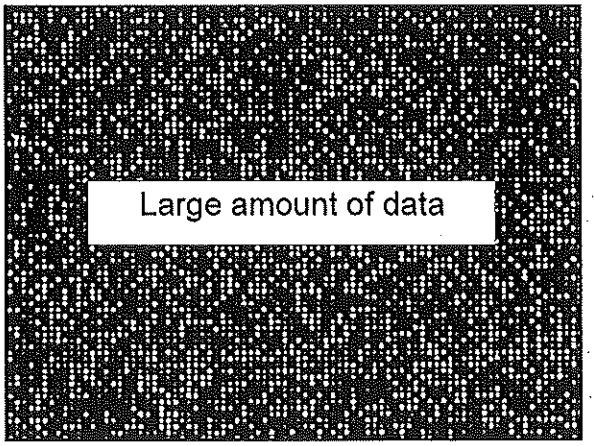
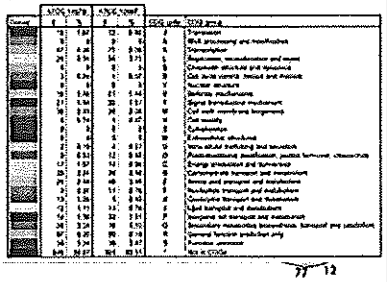


From molecule to management

- sequence
- annotation



- 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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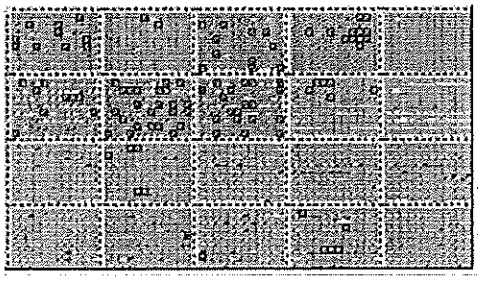
From molecule to management

phenotypic microarray



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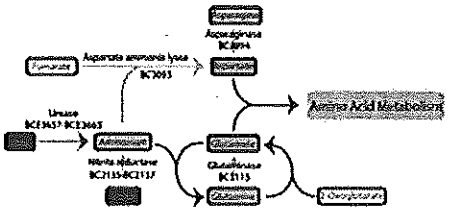
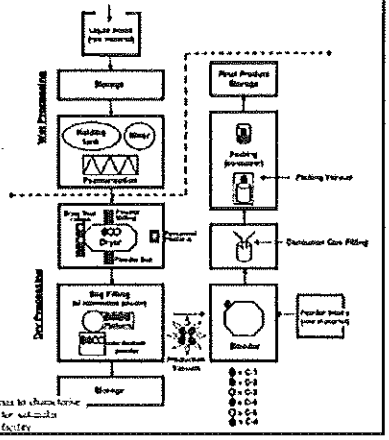
From molecule to management

understanding of mechanisms



in silico characteristics needs to be confirmed biochemically or phenotypically

Ecology



QMRA/QualRA

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

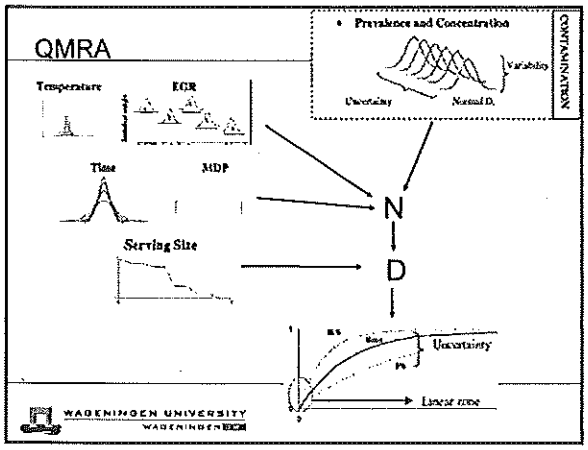
September 2003

USDA

QMRA

- N_0
- $mu(T_5) + T + t + N_{max}$
- N_t (cooking)
- serving size
- Dose
- $P_{ill}=1-exp(-r*D)$ + mortality

QMRA

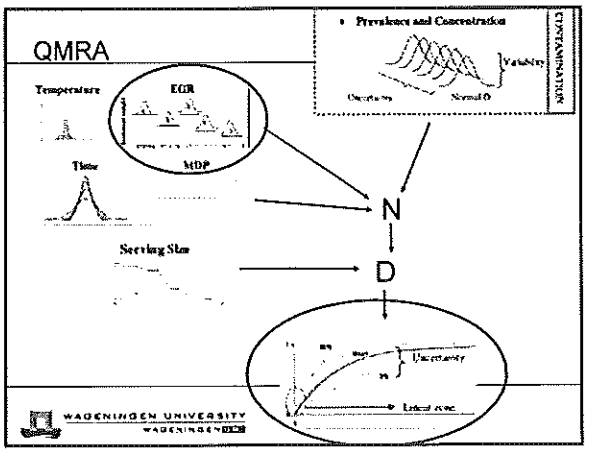


Kinetics

Multiple products / variability

Variability distribution of μ_{ref}

QMRA



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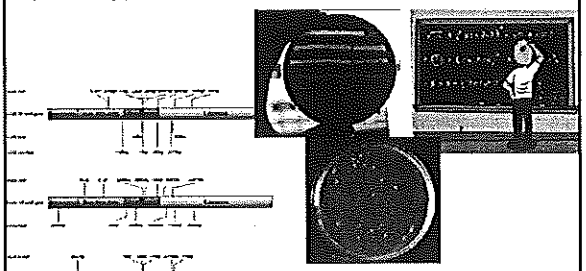
Getting insight with functional genomics, phenotype and mathematics



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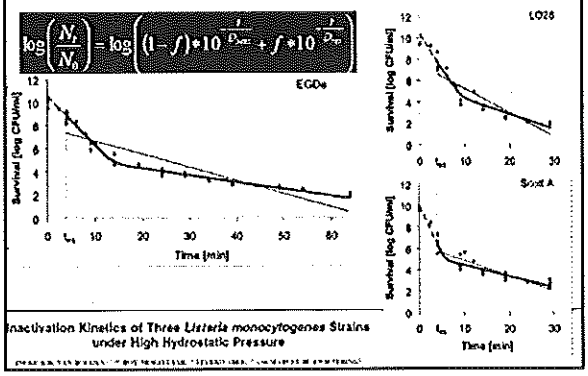
Non-linear inactivation described in HHP

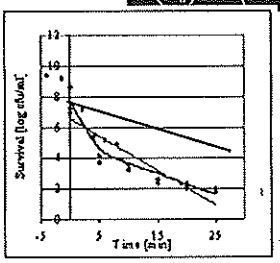
$$\log\left(\frac{N_t}{N_0}\right) = \log\left((1-f)\cdot 10^{-\frac{t}{D_{res}}} + f\cdot 10^{-\frac{t}{D_{tol}}}\right)$$



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

Investigate mechanism + quantitative *modelling*

$$\log\left(\frac{N_t}{N_0}\right) = \log\left((1-f)\cdot 10^{-\frac{t}{D_{res}}} + f\cdot 10^{-\frac{t}{D_{tol}}}\right)$$



Stable survivors

only for LO28 and Scott A

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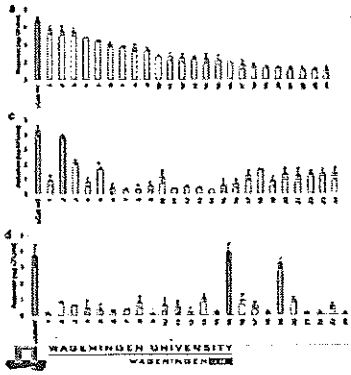
Robustness



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

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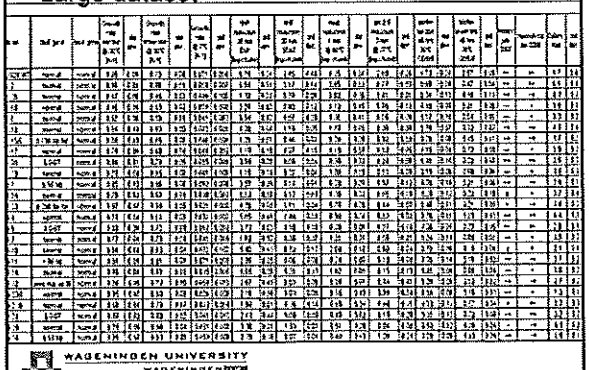
Large dataset



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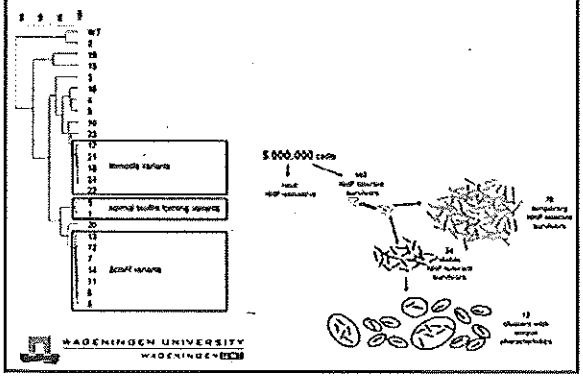
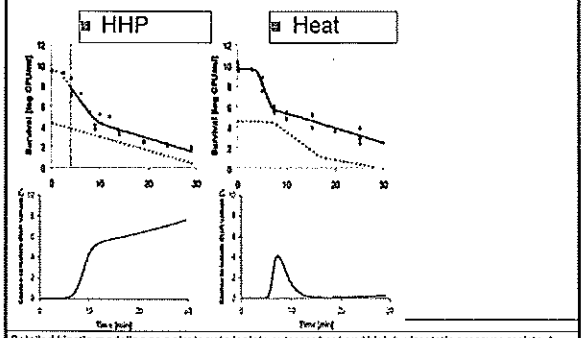
Cluster analysis



Specific finding or more widespread ?

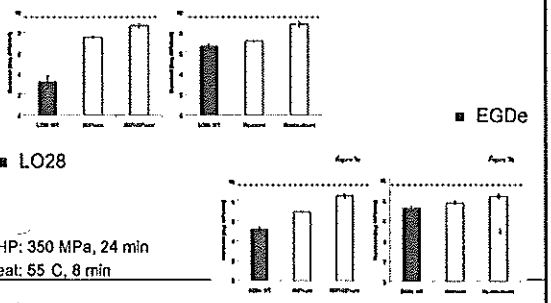
- All three strains non-linear inactivation
- No stable variants found for EGDe
- No stable variants found for heat inactivation

What is the fraction variants in time



Detailed kinetic modelling as a strategy to isolate extreme heat and high hydrostatic pressure resistant *Listeria monocytogenes* variants
Ineke K. H. Van Boeijen, Roy Moezelaar, Tjakko Abee, Marcel H. Zwietering

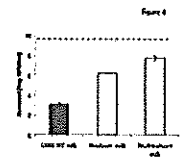
Strategy to find also heat stable and EGDe



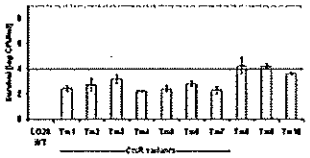
- EGDe
- LO28

HHP: 350 MPa, 24 min
Heat: 55 C, 8 min

..... And also for practical conditions



- Heat 72 C 6s
- Heat 72 C 15s



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

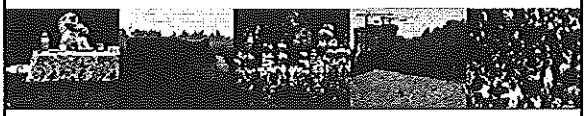
omics data (sequence, transcriptomes, ...)

Insight, prevalences and fractions

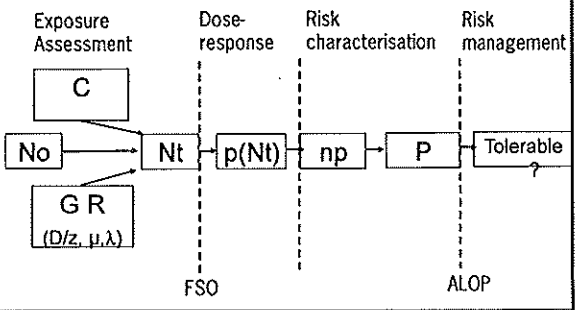
quantitative models

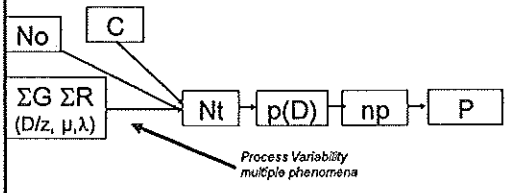
QMRA

Thank you



Overall analysis





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	$f_{0.05}$	Stable $f_{0.05}$	
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[a]	3.0×10^{-5}	1.2×10^{-5}	40% (33 out of 84)

[a]calculatated 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

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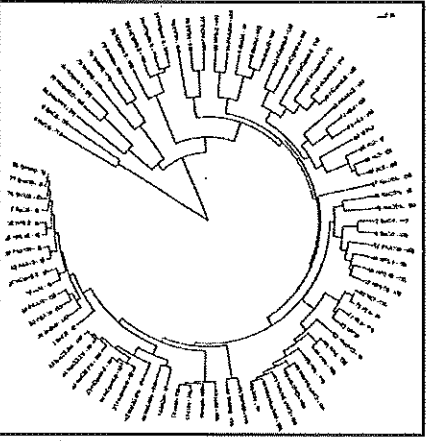
Population Diversity of *Listeria monocytogenes* LO28: Phenotypic and Genotypic Characterization of Variants Resistant to High Hydrostatic Pressure[a]



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