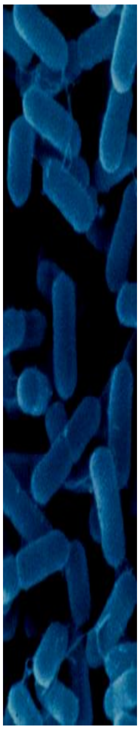
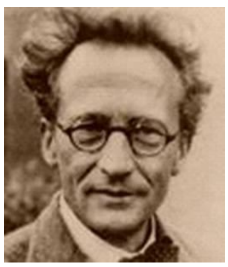


Individual cell heterogeneity in Predictive Food Microbiology: Challenges in predicting a “noisy” world

Kostas Koutsoumanis

Aristotle University of Thessaloniki
Dpt. Of Food Science and Technology
Lab of Food Microbiology and Hygiene



ERWIN SCHRODINGER

What is life? The Physical Aspect of the Living Cell.

Published in 1944

also a description of phenotypic “noise”

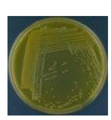
“.....All the physical and chemical laws that are known to play an important part in the life of organisms are of a statistical kind”

Food Safety Management

Traditional Food Safety Management approach was based on end-product testing

End Product Sampling

Is the product safe?



Yes

No

Food Safety

Qualitative (Discrete) variable

Food Safety Management

Traditional Food Safety Management approach was based on end-product testing

Early 90's

The HACCP system

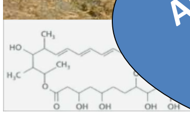
Food Safety

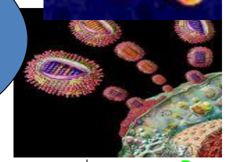
Qualitative (Discrete) variable

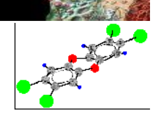
Food Safety Management

Traditional Food Safety Management approach was based on end-product testing

Food Safety Crisis of 90's







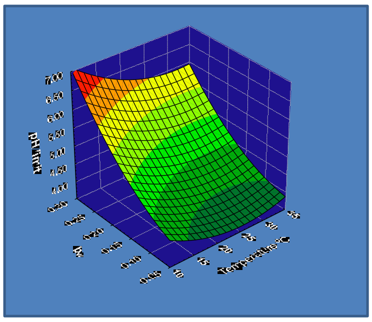
Antibiotics
E. coli
BSE
Salmonella
Listeria
SARS
Campylobacter

Need for change

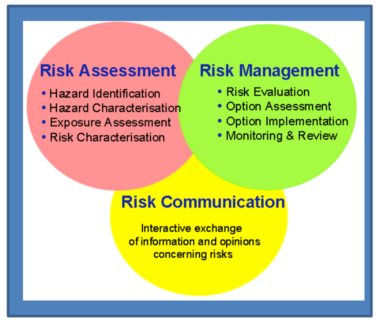
Food Safety Management

Development of new tools

Predictive Microbiology



Risk Assessment



Risk-based Food Safety Management

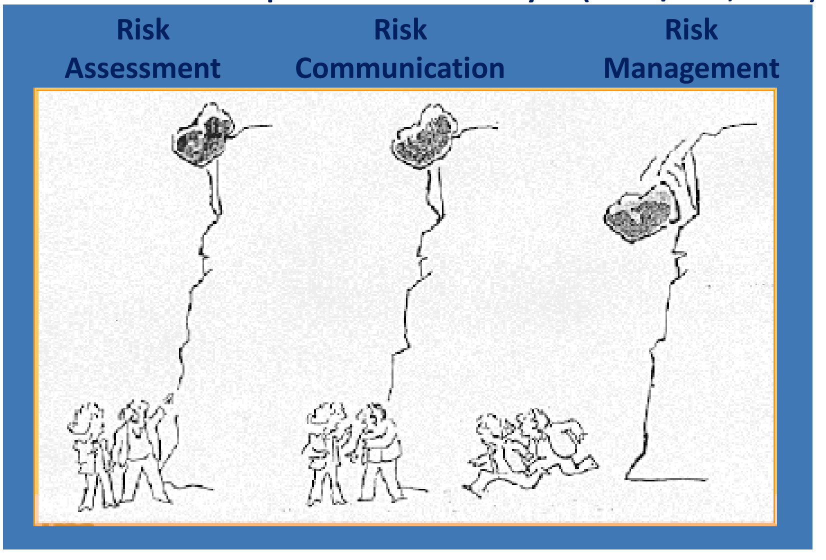
Food Safety Management

Risk Analysis

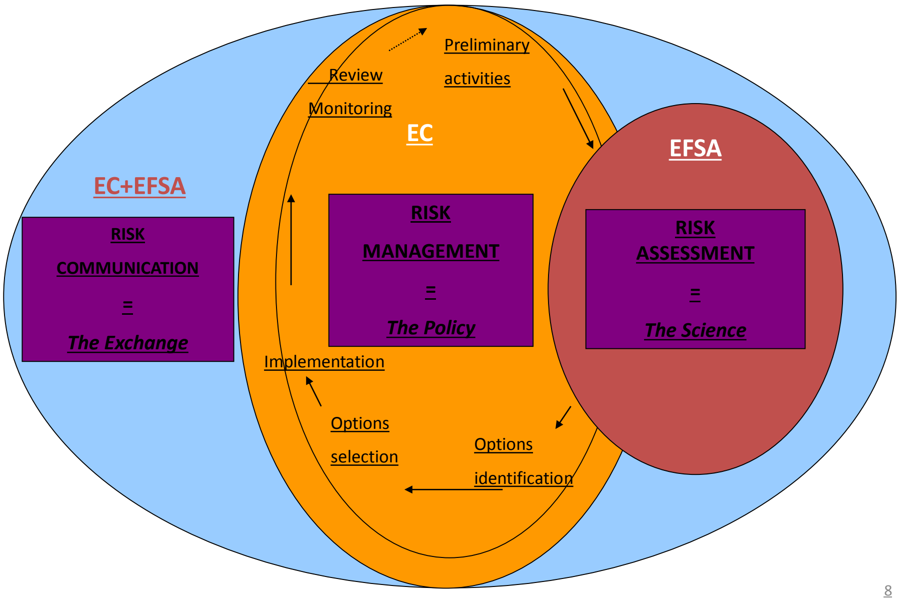
Food Safety Management

Risk Analysis

Risk Assessment is a component of Risk Analysis (WHO/FAO, 1995):



Risk Analysis in Europe



Microbial Risk Assessment

Microbiological risk assessment (MRA) provides a scientific description of food-borne risks related to the occurrence of pathogenic microorganisms in the whole food chain.

Microbiological Risk Assessment estimates

Number of cases of (a certain) illness per year per (e.g.) 100.000 persons in a given population caused by a certain micro-organism or group of microorganisms in a particular food or food type

The output of Risk Assessment

is a probability (Risk)

e.g 10^{-6}

Food Safety Management

Food Safety

Is the product safe? Yes/No

Qualitative (Discrete) variable

↓

Risk Assessment

↓

Quantitative (Continues) variable

What is the degree of product safety? Risk

Food Safety Management

100% Safety (zero risk) does not exists and should not be expected

Food Safety Management

Available online at www.sciencedirect.com

ScienceDirect

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Current Opinion in Food Science

Moving towards a risk-based food safety management

Konstantinos P Koutsoumanis and Zafiro Aspridou

CrossMark

Classical hazard-based approaches to food safety relying heavily on regulatory inspection and sampling regimes cannot sufficiently ensure consumer protection. It is now generally accepted that a modern food safety management system should link the hazards to public health and be based on prevention rather than end product testing and control. The last decade food safety management at international level has been moved towards a more risk-based approach to food safety control with regulators around the world adopting the risk analysis framework as the basis for their decision-making. This review paper presents an overview of the structure and function of a risk based food safety management and the interaction between risk managers, risk assessors and stakeholders.

Address
Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

Corresponding author: Koutsoumanis, Konstantinos P (kkoutsou@agro.auth.gr)

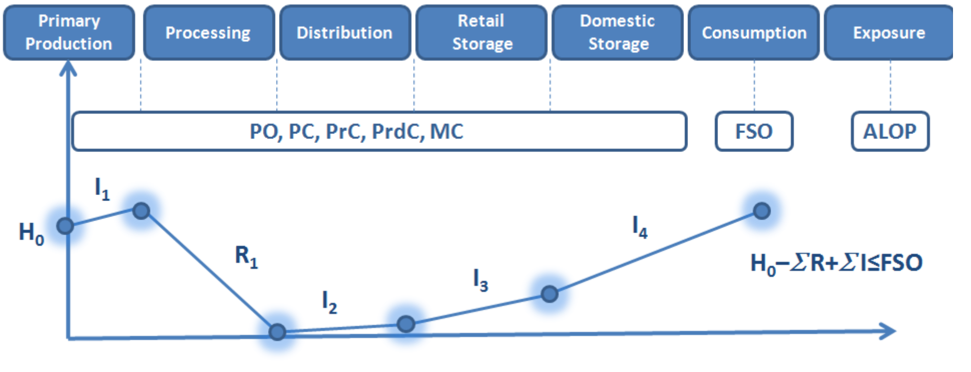
(WTO) suggested for the first time, in the mid-1990s, a risk assessment basis for food safety. SPS Agreement introduced the term 'appropriate level of health protection' (ALOP) as the 'Level of protection deemed appropriate by the member (country) establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory'. With ALOP, WTO changed the question 'is the food safe?' to 'what is the level of product safety?' and transformed food safety from a discrete (safe/unsafe) to a continuous (risk) variable recognizing that 100% safety (or zero risk) does not exist. The European Commission followed with Regulation (EC) 178/2002 which clearly states that food safety should generally be founded on science using the Risk Analysis framework [2]. In 2003, the Codex Alimentarius Commission adopted the Principles for Food Safety and Risk Analysis to be used in the Codex framework. During the last decade, considerable progress has been made in developing a framework and principles for risk analysis with many guidance documents for the application of risk management and risk assessment by governments [3–6].

Food Safety Management

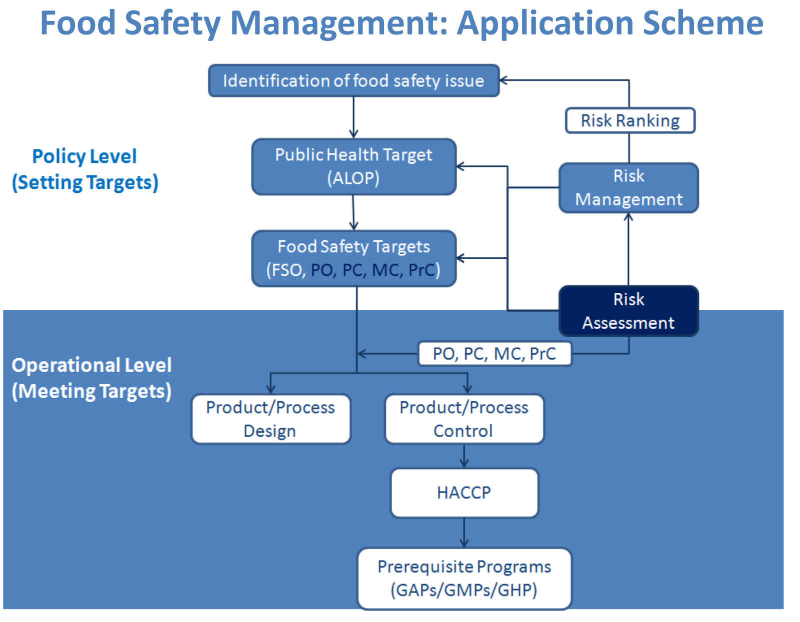
Food Safety Management: System structure

Food Safety Management

Food Safety Management: Application Scheme



Food Safety Management



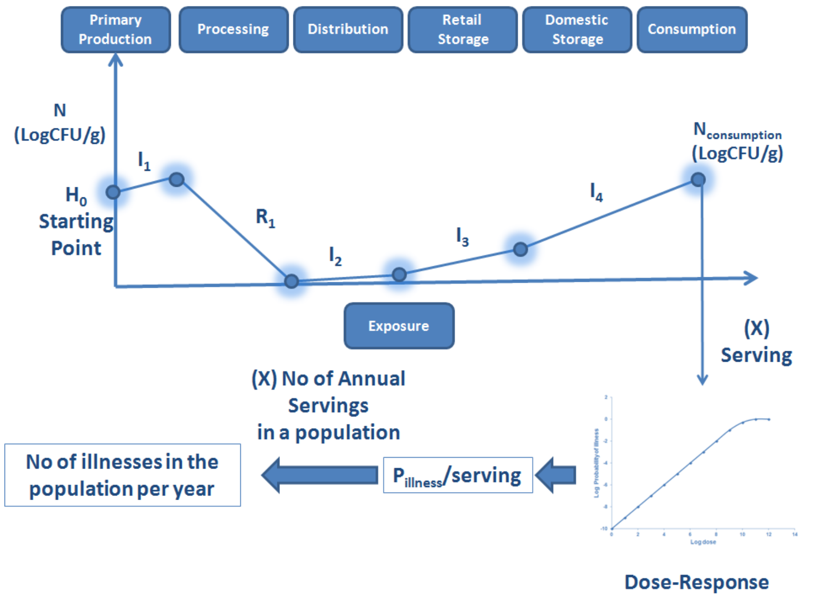
Risk Assessment

"Microbiological risk assessment" is a structured systematic process to support food safety risk management decisions

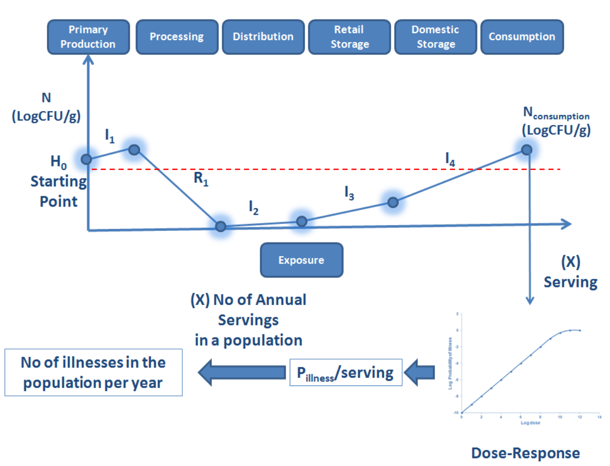
The goals are:

- to provide an estimate of the level of health burden in a given population from a hazardous microorganism or group of microorganisms in a particular food
- To evaluate and propose mitigation options in order to reduce the risk

Risk Assessment Infograph



Risk Assessment Infograph



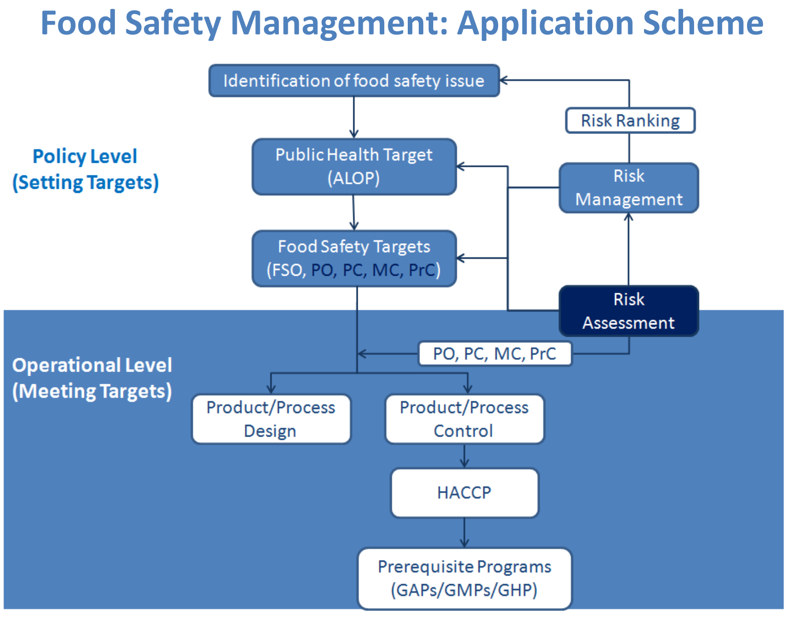
Evaluation of Mitigation Strategies

'what if' we regulate a limit at the time of consumption?

The FBOs should take actions and assure meeting the limit

The authorities should inspect the

Food Safety Management



Example

Risk analysis applied for Covid-19

Risk Assessment

Epidemiological data and models

Kermack-McKendrick Model

(a susceptible-infected-recovered or SIR model with S, I, and R representing the 3 compartments)

$$(1) \quad \frac{dS}{dt} = -\frac{\beta I}{N} S$$

$$(2) \quad \frac{dI}{dt} = \frac{\beta I}{N} S - \gamma^{*} I$$

R0=β*S/γ

$$(3) \quad \frac{dR}{dt} = \gamma^{*} I$$

$$N = S + I + R.$$

Example

Risk analysis applied for Covid-19

Risk Assessment

Epidemiological data and models

Objectives

- **Predict future number of covid-19 infections, hospitalizations and deaths**

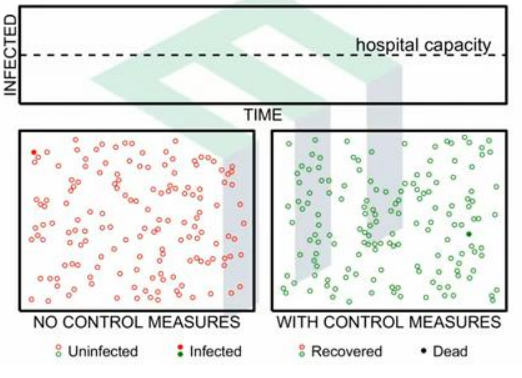
- **To evaluate and propose mitigation options in order to reduce the risk**

Example

Risk analysis applied for Covid-19

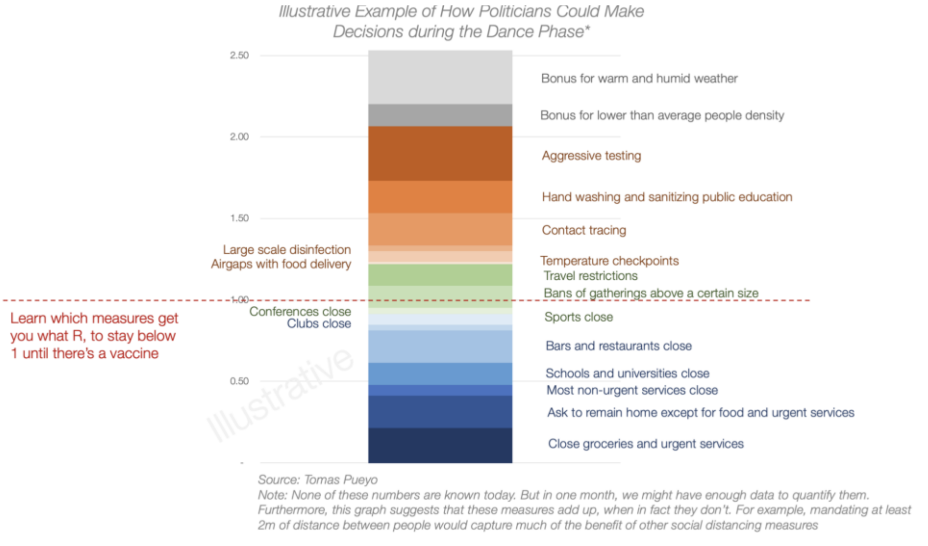
Risk Assessment

Epidemiological data and models



Example

Risk analysis applied for Covid-19



Microbial Risk Assessment

Important Aspects of Risk Assessment

Variability represents a true heterogeneity of the population that is a consequence of the physical system and irreducible (but better characterized) by further measurements.

Uncertainty represents the lack of perfect knowledge of a parameter value, which may be reduced by further measurements.

Exposure Assessment

Variability (Example)

We all want to move to the 5th floor using the elevator in groups of 5 (randomly selected) people
The weight limit of the elevator is 480 kg

Estimate the chance of exceeding the weight limit

Deterministic method (variability is not taken into account)

Average individual weight=70 kg

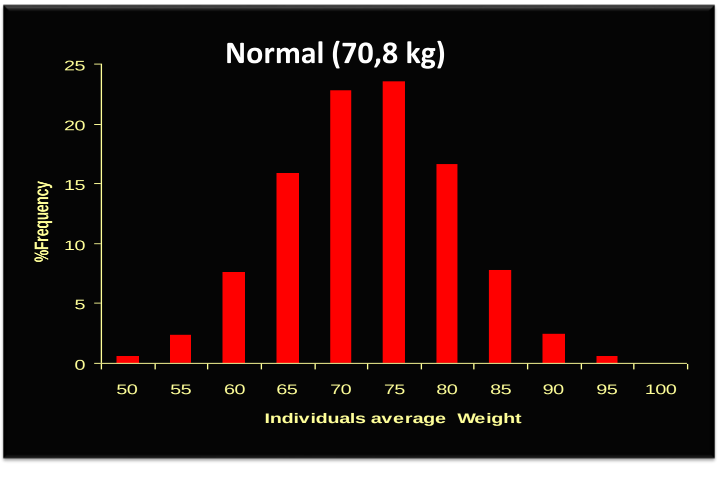
5 persons x 70 =350 kg<480 kg

The weight limit is not exceeded

Exposure Assessment

Variability (Example)

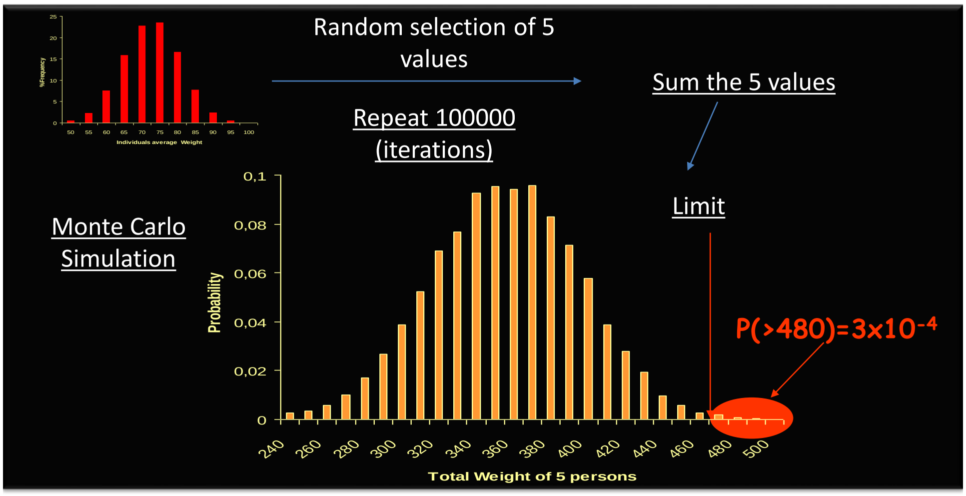
Stochastic method (variability is taken into account)



Exposure Assessment

Variability (Example)

Stochastic method (variability is taken into account)



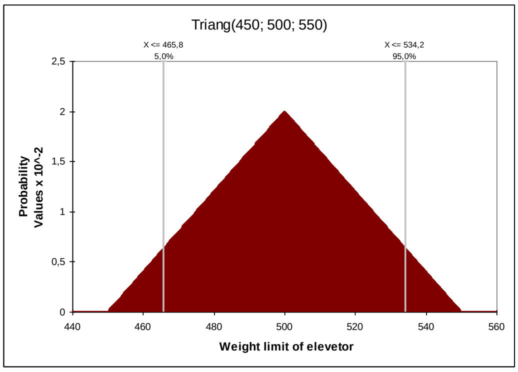
Exposure Assessment

Uncertainty (Example)

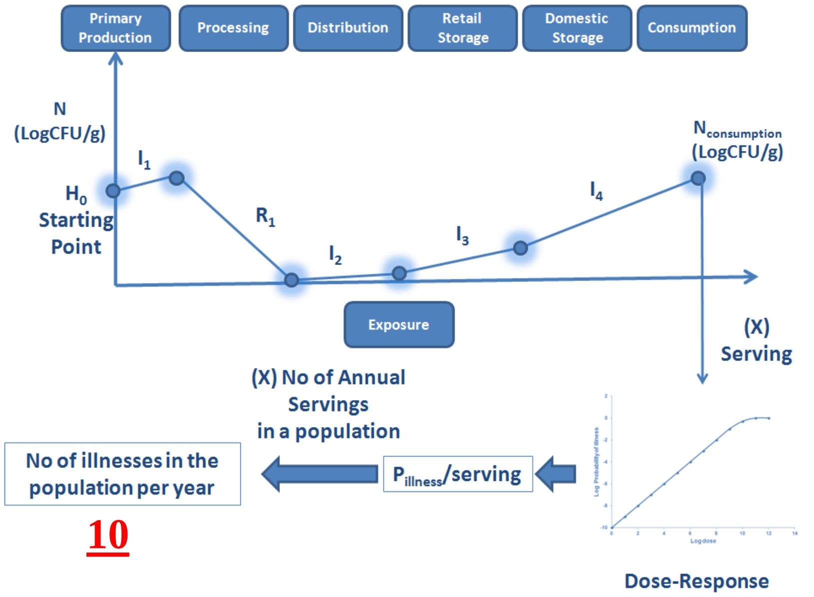
Stochastic method

Uncertainty: We don't know the weight limit of the elevator

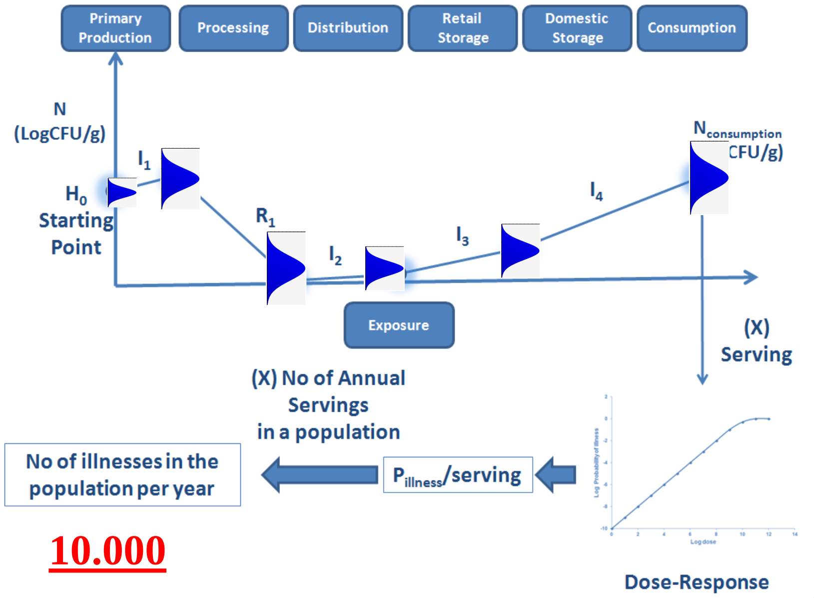
Expert Opinion: Min:450, Max:550 Most likely:500



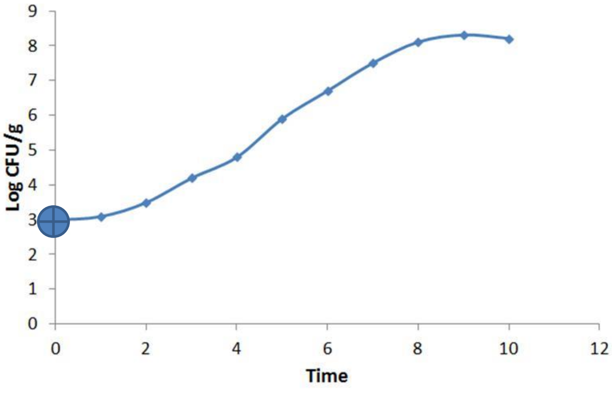
Risk Assessment Infograph



Risk Assessment Infograph

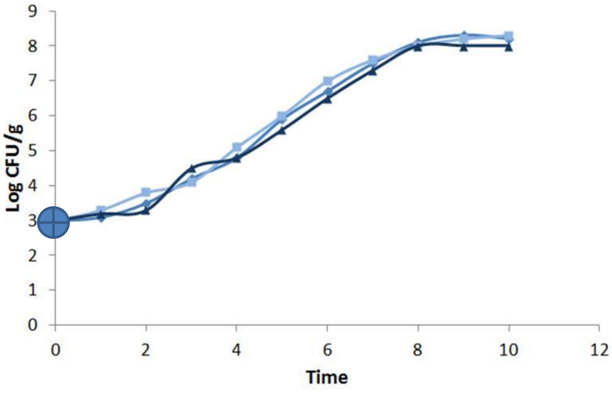


Intro: Microbial behavior



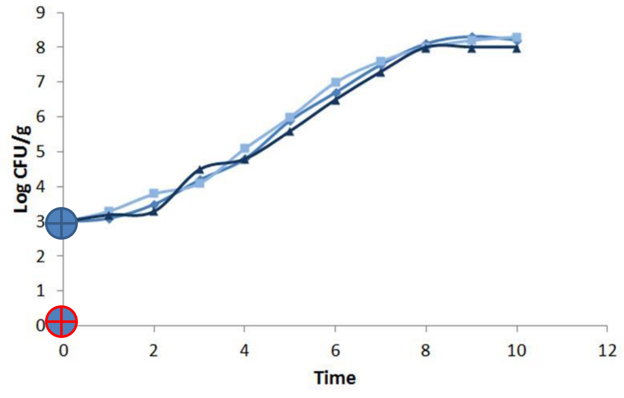
For many years Food Microbiology experiments were based on large microbial populations…….

Intro: Microbial behavior



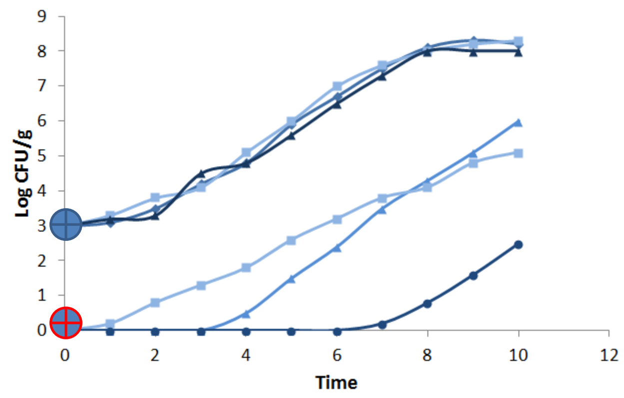
The advantage of using large microbial populations is that microbial behavior is reproducible…….

Intro: Microbial behavior



The disadvantage is that it is not realistic…. Contamination of foods with pathogens occurs at the level of one or few cells…

Intro: Microbial behavior



For small microbial populations reproducibility is lost and microbial behavior is more variable

The big change………

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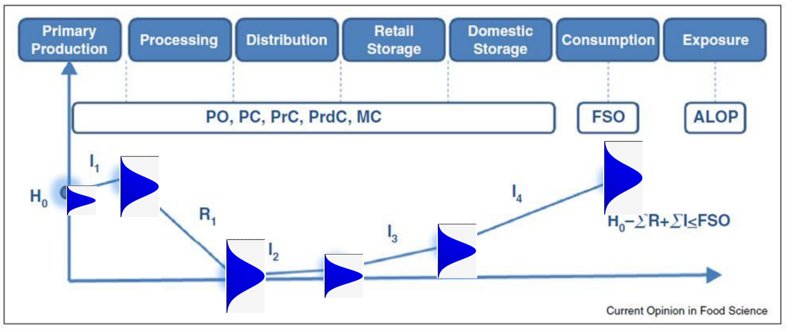
Current Opinion in Food Science

Moving towards a risk-based food safety management

Konstantinos P Koutsoumanis and Zafiro Aspridou



Safety management targets applied in the food chain.

www.sciencedirect.com Current Opinion in Food Science 2016, 12:36–41

Risk=Probability x Severity

Variability is of great importance in Risk Assessment

Variability sources in microbial behavior ………

Available online at www.sciencedirect.com
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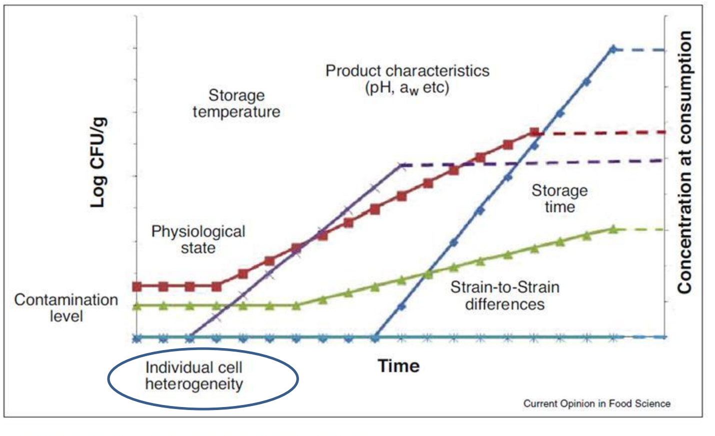
Current Opinion in Food Science

Last developments in foodborne pathogens modeling

Konstantinos P Koutsoumanis[1], Alexandra Lianou[2] and Maria Gougouli[3]

Figure 1



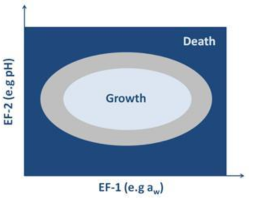
Sources of variability affecting microbial growth and exposure assessment.

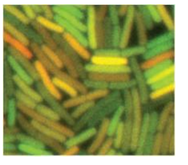
www.sciencedirect.com Current Opinion in Food Science 2016, 8:89–98

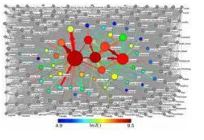
Individual cell-based Food Microbiology: Insights into a “noisy” World

Presentation outline

- **Behavioral (phenotypic) noise**
  - -Growth
  - -Inactivation
  - -Interface
- **Molecular noise**
- **Role of noise in cell function**
- **Future Challenges**







Microbial Growth

AEM

Stochasticity in Colonial Growth Dynamics of Individual Bacterial Cells

Konstantinos P. Koutsoumanis, Alexandra Lianou
Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

Time-lapse microscopy method for monitoring colonial growth of single cells.

The quality of the images was improved by developing an auto focus procedure with an Extended Depth of Focus (EDF) system using the ScopePro module of ImageProPlus software.

Each final image was a result of 20-30 images captured in different z-axis planes. The EDF system allowed the combination of the z-stack images of multi-level focal planes into a single in-focus image.

Microbial Growth


AEM
Journals.ASM.org

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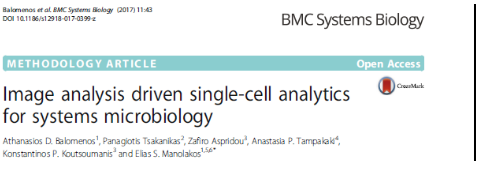
Time-lapse microscopy method for monitoring colonial growth of single cells (Salmonella).

Extracted Info:

Number and properties of cells in a growing colony (division tine, elongation rate, size etc)

Microbial Growth


Balomenos et al. BMC Systems Biology (2017) 11:43
DOI 10.1186/s12918-017-0399-z

BMC Systems Biology

METHODOLOGY ARTICLE Open Access

Image analysis driven single-cell analytics for systems microbiology

Athanasios D. Balomenos[1], Panagiotis Tsakanikas[2], Zafiro Aspridou[3], Anastasia P. Tampakaki[4], Konstantinos P. Koutsoumanis[3] and Elias S. Manolakos[1,5,6*]

Visualization of the properties of cells in a growing colony for spatial analysis

Microbial Growth

AEM

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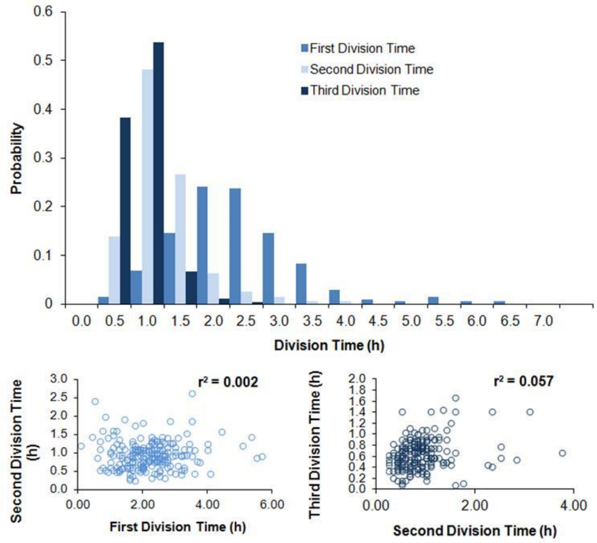
Division times of Salmonella individual cells

Both the mean and the spread of division time distributions decreased with generations

*The poor correlation indicates no intergenerational "memory" related to the time required for division.



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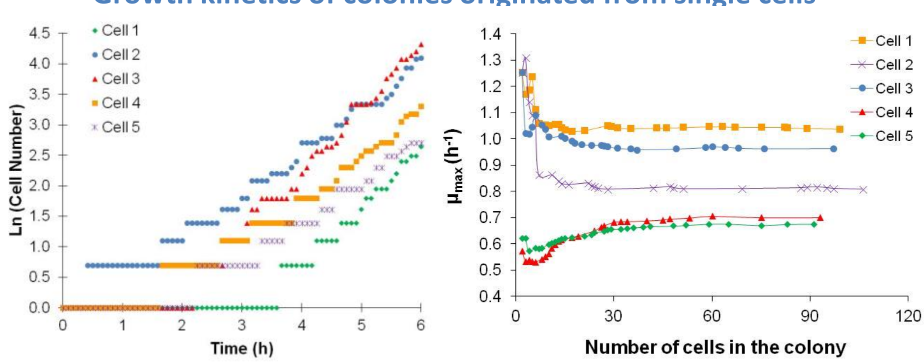
Growth kinetics of colonies originated from single cells



*when the number of cells in a microcolony exceeds 20 to 25, the growth rate reaches a constant value, which varies significantly among microcolonies

*The heterogeneity in colonial growth rate cannot be explained by the statistics of individual cells

Microbial Growth

AEM

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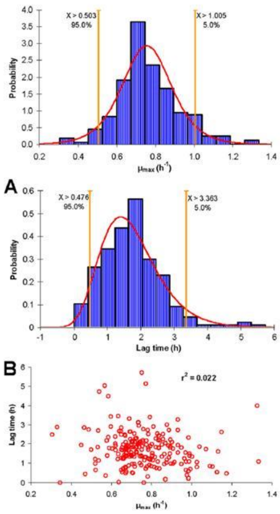
Individual cell-based predictive food microbiology



$$N_t = (N_0 - N_g) + \sum_1^{N_g} \begin{cases} 1 & \text{for} \quad t \leq \lambda_i \\ e^{\mu_{\max\ i}\,(t - \lambda_i)} & \text{for} \quad t > \lambda_i \end{cases}$$

The model describes the growth of a bacterial population, initially consisting of N_0 cells, over time as the sum of cells in each of the N_0 imminent microcolonies originating from a single cell.

Microbial Growth

AEM

Stochasticity in Colonial Growth Dynamics of Individual Bacterial Cells

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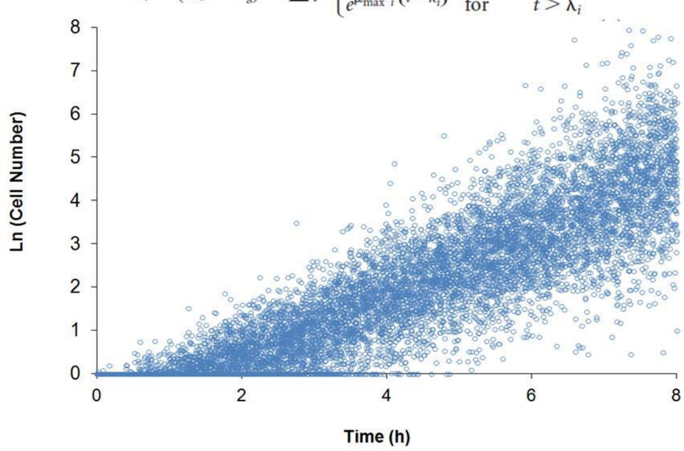

Microbial Growth

AEM
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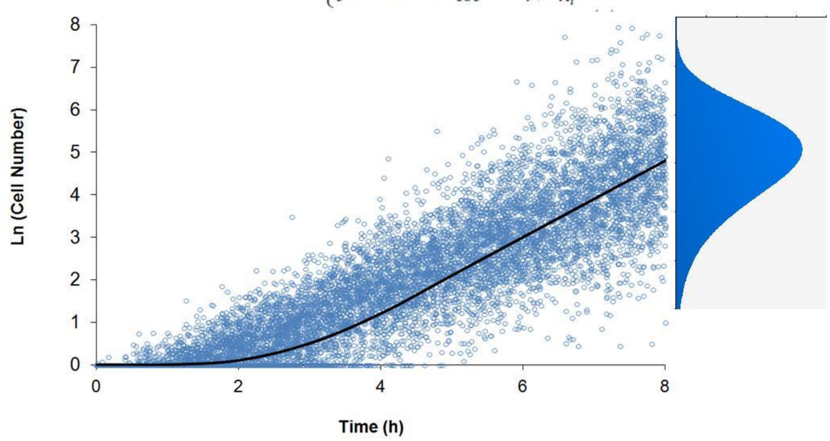

Microbial Growth

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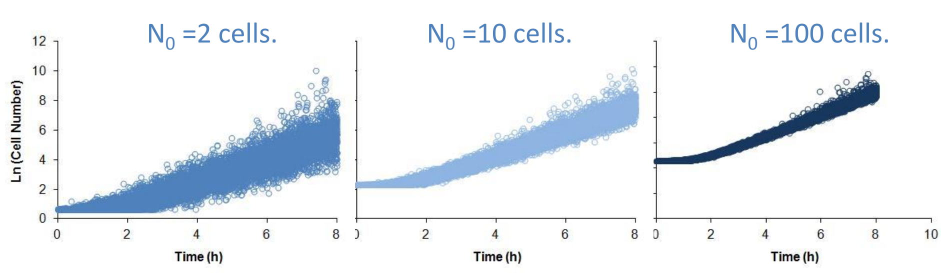


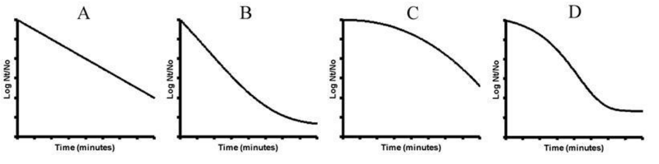
*For bacterial populations with N_0 of>100 cells the variability is masked and the system seems to behave deterministically, even though the underlying law is stochastic.

Microbial Inactivation

➢For more than 100 years microbial inactivation is described with the deterministic **D-value approach** based on the study of **Bigelow in 1921**

➢An increased number of studies have reported deviations from log-linear death and attempted to incorporate them in mathematical models

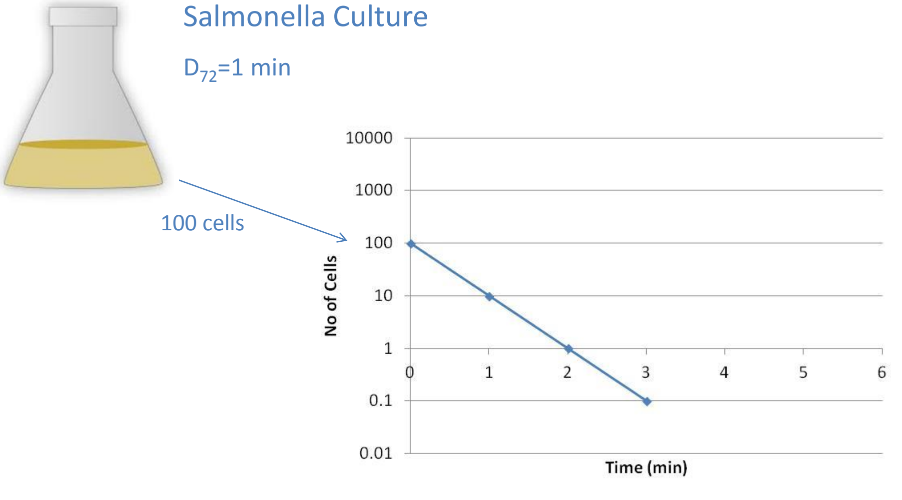
➢Despite the progress in this area, the majority of microbial inactivation models are based on deterministic approaches without taking into account the variability in microbial responses



The problem of the deterministic approach….

Salmonella Culture

D_{72}=1 min



*All 100 cells taken from the flask will be died after 3 min

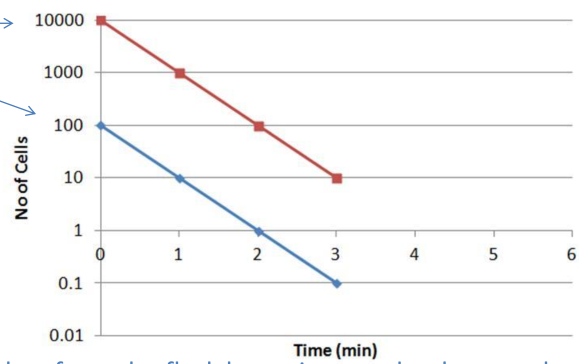
The problem of the deterministic approach….

Salmonella Culture

D_{72}=1 min

10.000 cells

100 cells



*10 out of 10.000 cells taken from the flask have time-to-death more than 3 min

*When I take 100 cells from the flask there is a probability of taking some of those ten cells

Microbial Inactivation


Food Microbiology 45 (2015) 216–221

Contents lists available at ScienceDirect

Food Microbiology

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

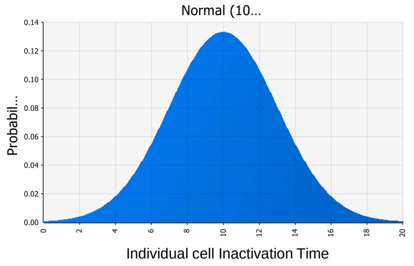
Individual cell heterogeneity as variability source in population dynamics of microbial inactivation

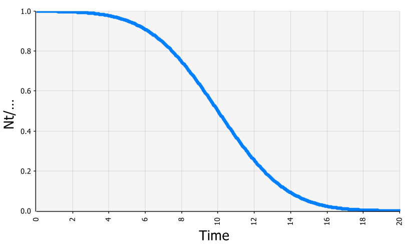
Zafiro Aspridou, Konstantinos P. Koutsoumanis

Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

➢Microbial inactivation is considered as a probability distribution

➢The inactivation curve in the form Nt/N0 vs time is the cumulative descending distribution of the individual cell inactivation times.





Microbial Inactivation as probability distribution…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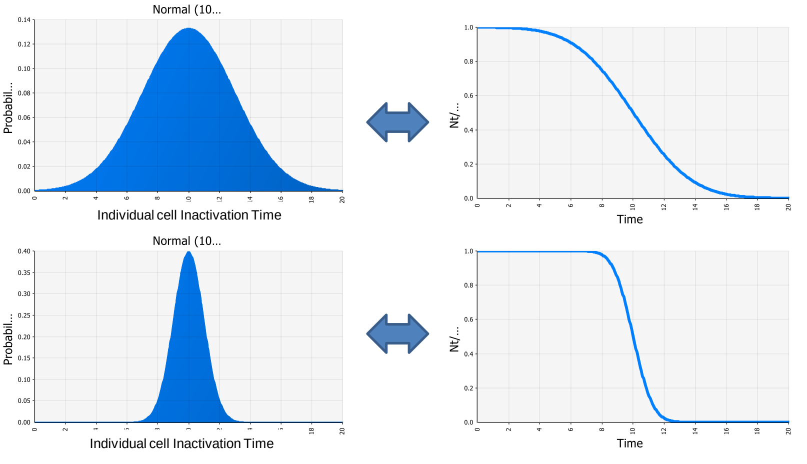
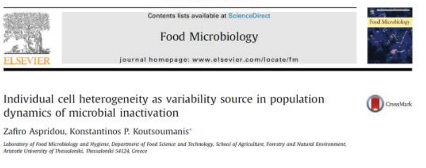
➢The **inactivation rate** of the curve is defined by **the variance (st. dev)** of the individual cell inactivation times distribution.



Microbial Inactivation as probability distribution…



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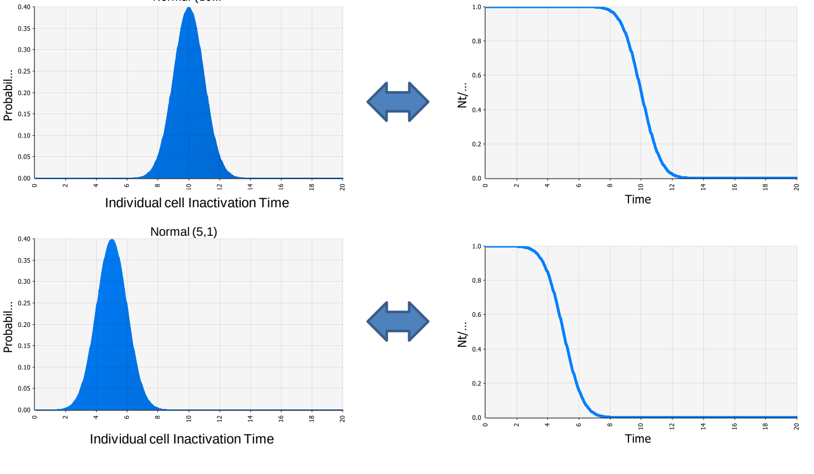
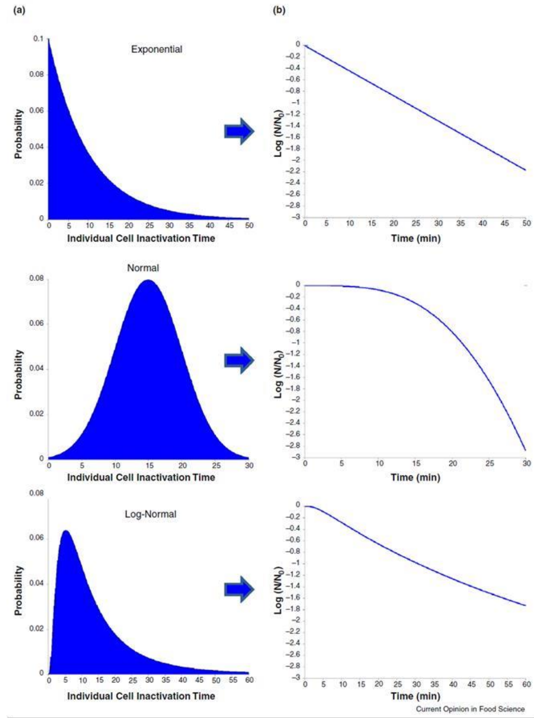
➢The **"shoulder" (lag)** of the curve is defined by **position (mean)** of the individual cell inactivation times distribution.



Microbial Inactivation as probability distribution…



- ➢The **shape** of the inactivation curve is defined by **type** of the individual cell inactivation times distribution.
- ➢All observed shapes of inactivation curve can be described by **a single or a combination of probability distributions**

Microbial Inactivation as probability distribution…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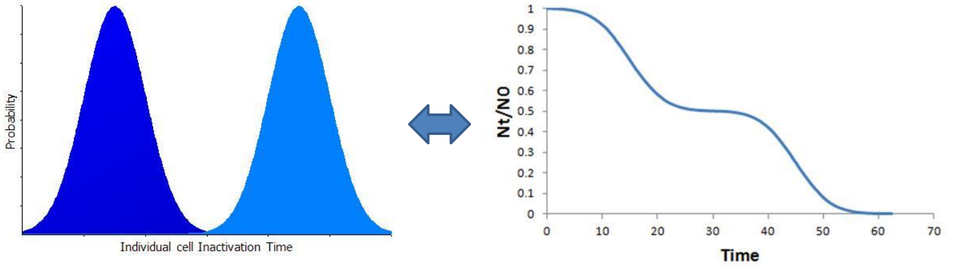
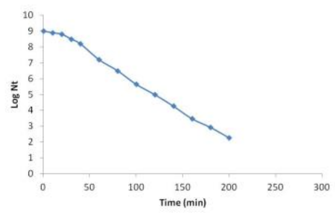
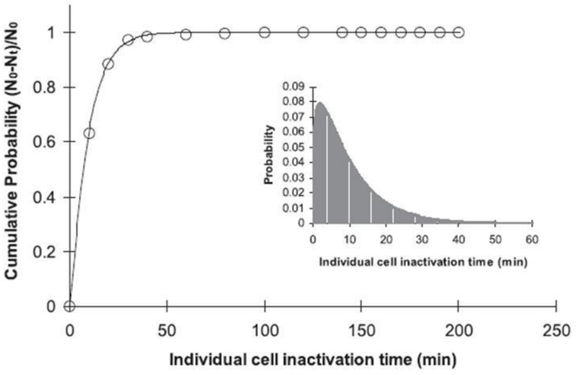
- ➢A double sigmoidal inactivation curve can be explained by a heterogeneous population in which individual cell inactivation times follow two distributions.



Microbial Inactivation as probability distribution…

➢individual cell inactivation times distribution.



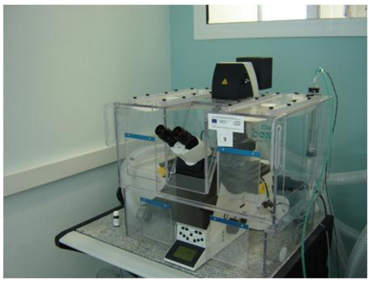


*the variability of individual cell inactivation times can be evaluated indirectly based on the cumulative data from the inactivation curve.

*this results to a less accurate description of the distribution, especially with regard to the tailing part which is very important for the variability in the population behavior.

Microbial Inactivation as probability distribution…

Time-lapse microscopy for monitoring individual cell time-to-death



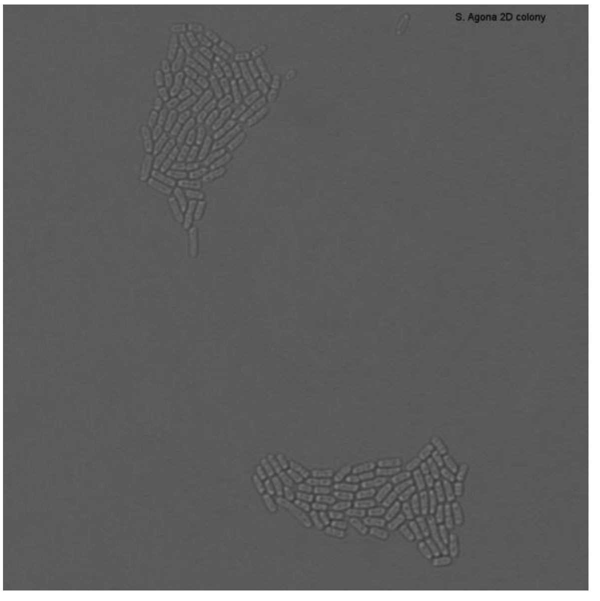
Confocal Laser Scanning Microscopy

Propidium Iodide- Fluorescent dye- Cells with damaged membrane
Dead cells –red
In house image analysis program

Strain	*Salmonella enterica* ser. Agona
Media	Tryptone Soy Broth / Agar
Conditions	✓pH 3.5 (lactic acid) ✓26% w/w NaCl
Method	Plate count/ Microscopy

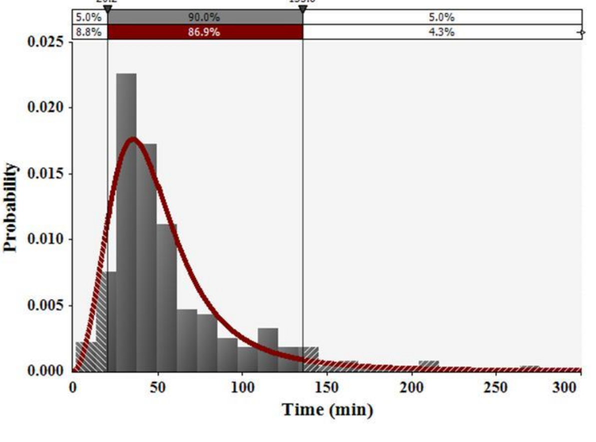
Microbial Inactivation as probability distribution…

Time-lapse microscopy for monitoring individual cell time-to-death

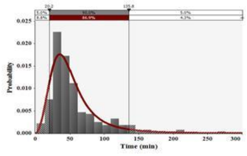


Microbial Inactivation as probability distribution…

Time-lapse microscopy for monitoring individual cell time-to-death
inactivation time



Stochastic modeling of microbial inactivation based on individual cell inactivation times distribution



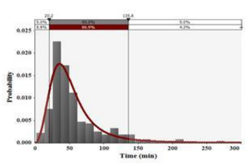
The distribution is used to predict the microbial inactivation of an initial level N_0 using Monte Carlo simulation

with

the number of iterations in each simulation being equal to N_0

the number of simulations representing the variability of the population inactivation behavior.

Stochastic modeling of microbial inactivation based on individual cell inactivation times distribution



Example: N_0=10 cells

1.Randomly select 10 (=N_0) values from the distribution

71.18
66.97
111.20
34.63
93.94
27.59
42.84
54.89
52.64
23.83

2.Rank the values

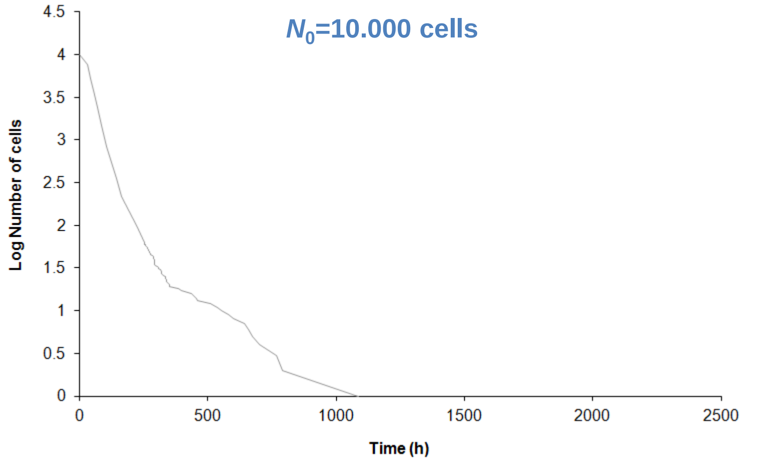
23.83
27.59
34.63
42.84
52.64
54.89
66.97
71.18
93.94
111.20

3.Built Inactivation Curve

Time	Nt
0	10
23.83	9
27.59	8
34.63	7
42.84	6
52.64	5
54.89	4
66.97	3
71.18	2
93.94	1
111.20	0

4.Run many simulations to describe variability

Stochastic modeling of microbial inactivation based on individual cell inactivation times distribution



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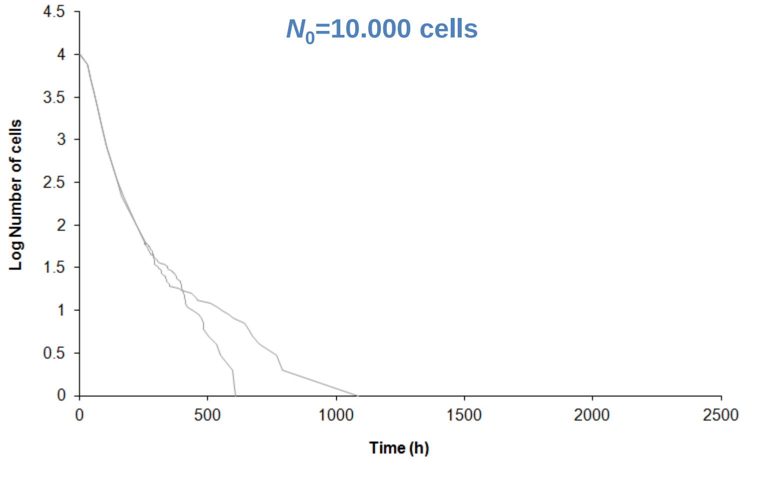
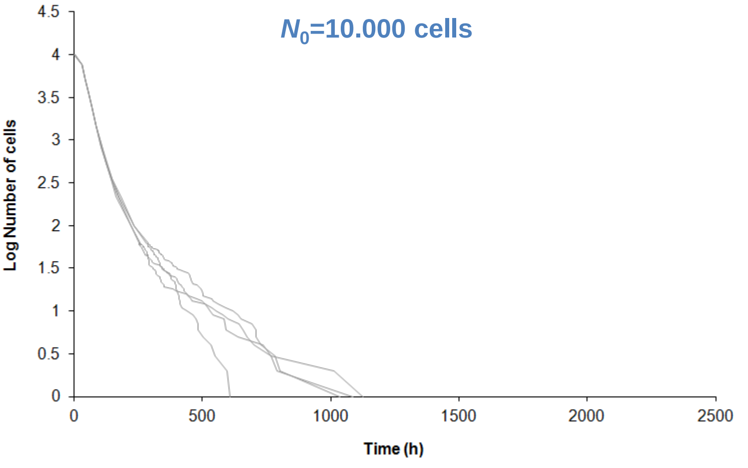


Stochastic modeling of microbial inactivation based on individual cell inactivation times distribution



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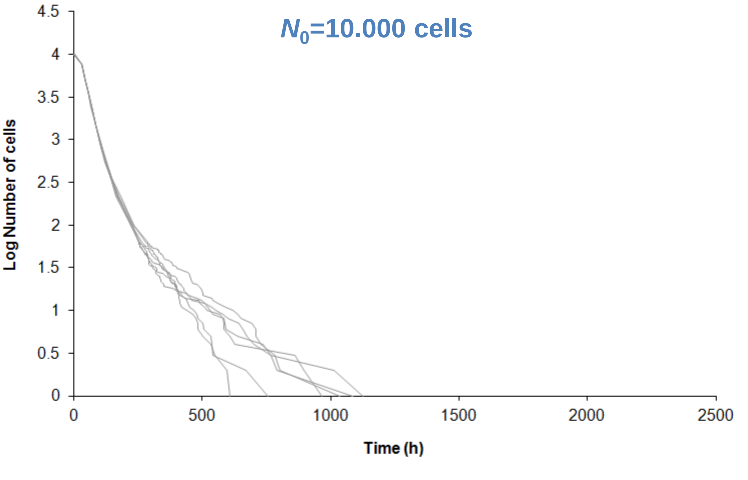

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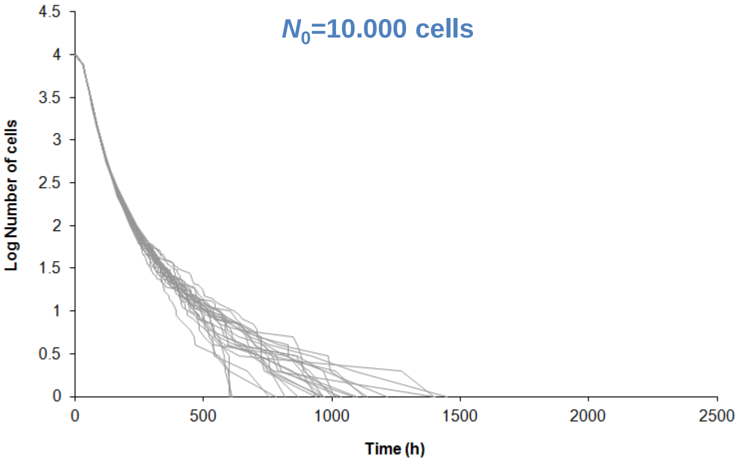

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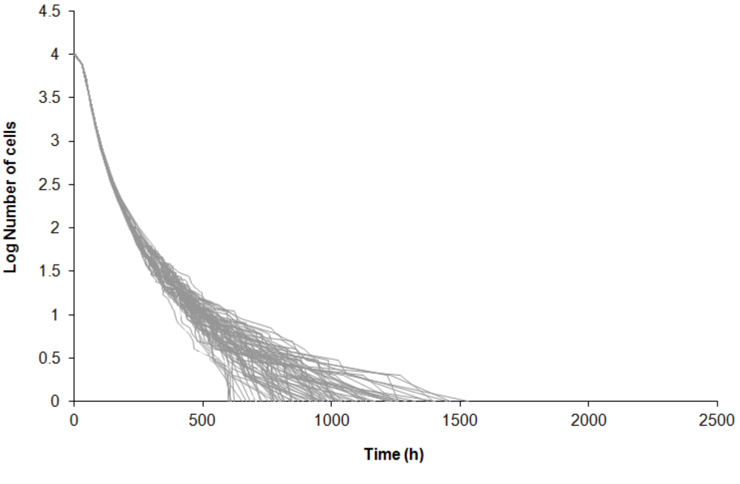

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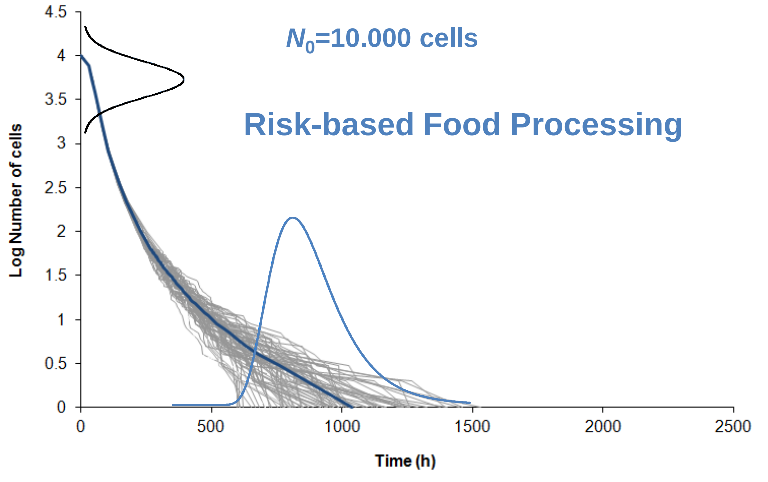
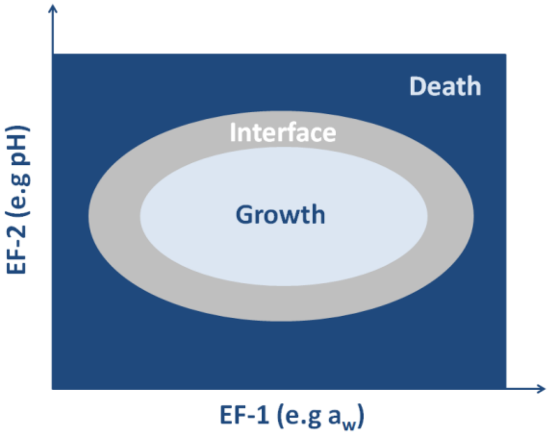


Interface between Microbial Growth and Inactivation



....study, understand and predict microbial behavior in the grey zone between growth, and death...

Interface


International Journal of Food Microbiology 128 (2008) 116–121

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

ELSEVIER

A study on the variability in the growth limits of individual cells and its effect on the behavior of microbial populations

K. Koutsoumanis *

Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, Faculty of Agriculture, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece

Inoculation of TSA with 100 cells of Salmonella

0.50%	2.50%	3.50%	4.50%	5.50%	6.50%
NaCl					

Interface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Inoculation of TSA with 100 cells of Salnonella

96*	91	65	32	10	0
0.50%	2.50%	3.50%	4.50%	5.50%	6.50%
NaCl					

***Colonies formed after incubation**

Interface

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Inoculation of TSA with 100 cells of Salnonella

96*	91	65	32	10	0
0.50%	2.50%	3.50%	4.50%	5.50%	6.50%
NaCl					

*Colonies formed after incubation

Inoculation of TSA with 10.000 cells of Salnonella

*	*	*	*	*	3
0.50%	2.50%	3.50%	4.50%	5.50%	6.50%
NaCl					

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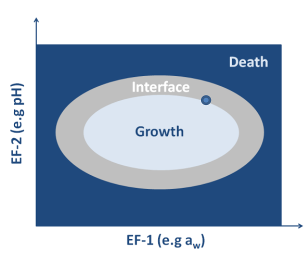

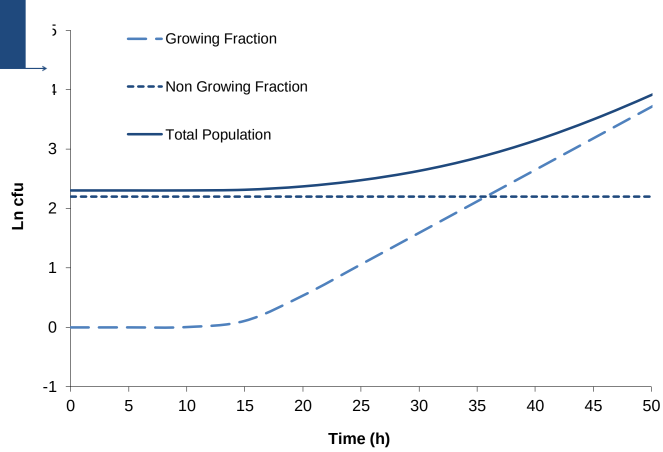

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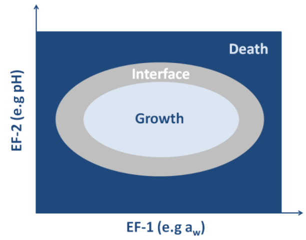

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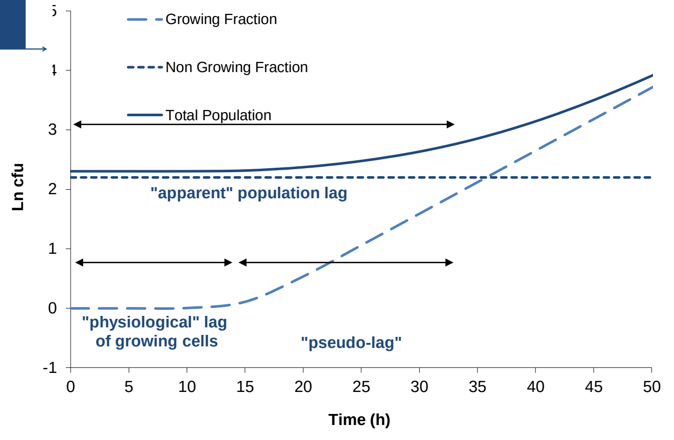

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International Journal of Food Microbiology 285 (2018) 103–109
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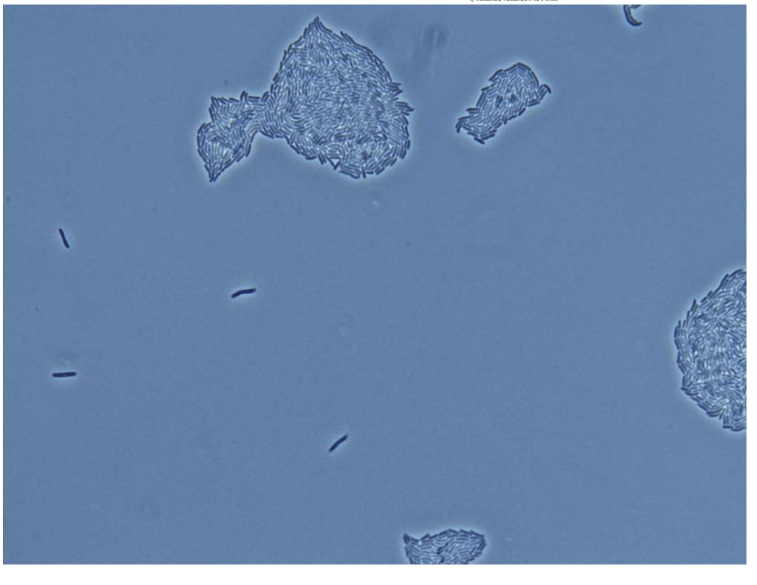

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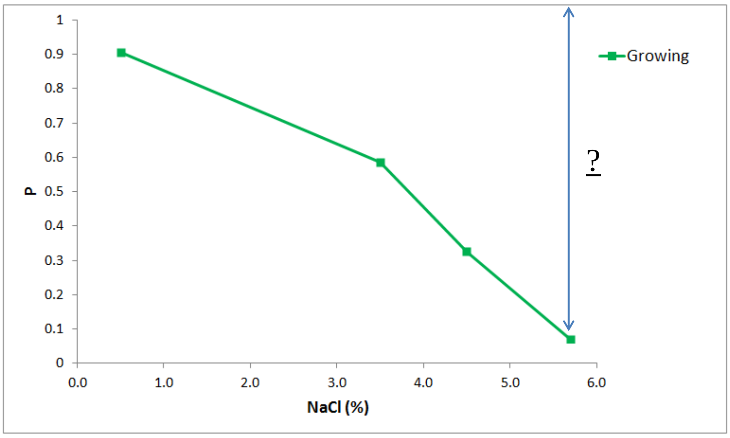
	%NaCl			
	0.5	**3.5**	**4.5**	**5.7**
Total No of cells	277	472	413	435
dividing cells	251	276	134	30
not dividing cells	26	186	279	405
Pg	**0.91**	**0.58**	**0.32**	**0.07**



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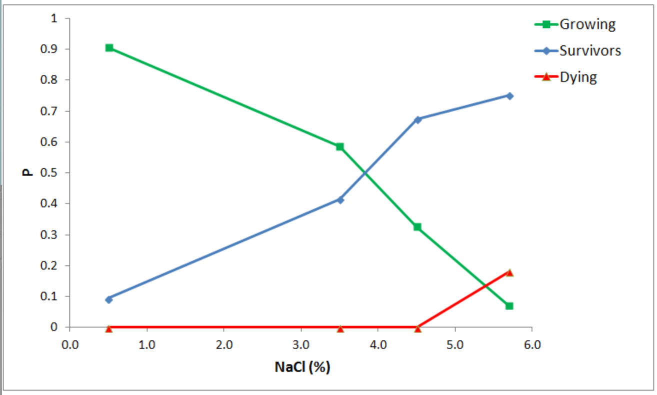

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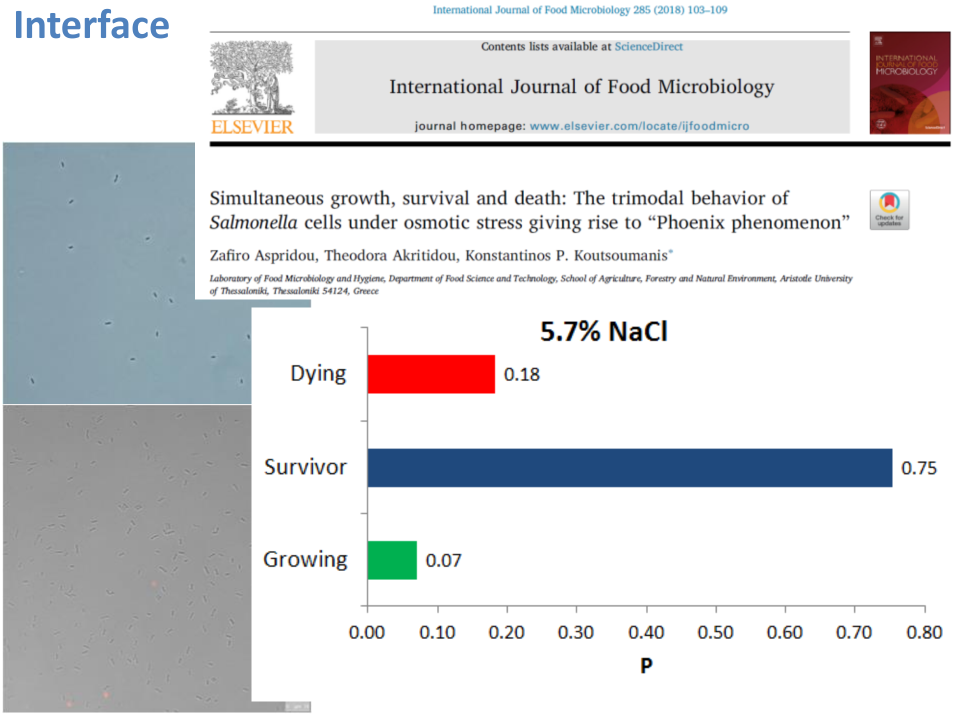
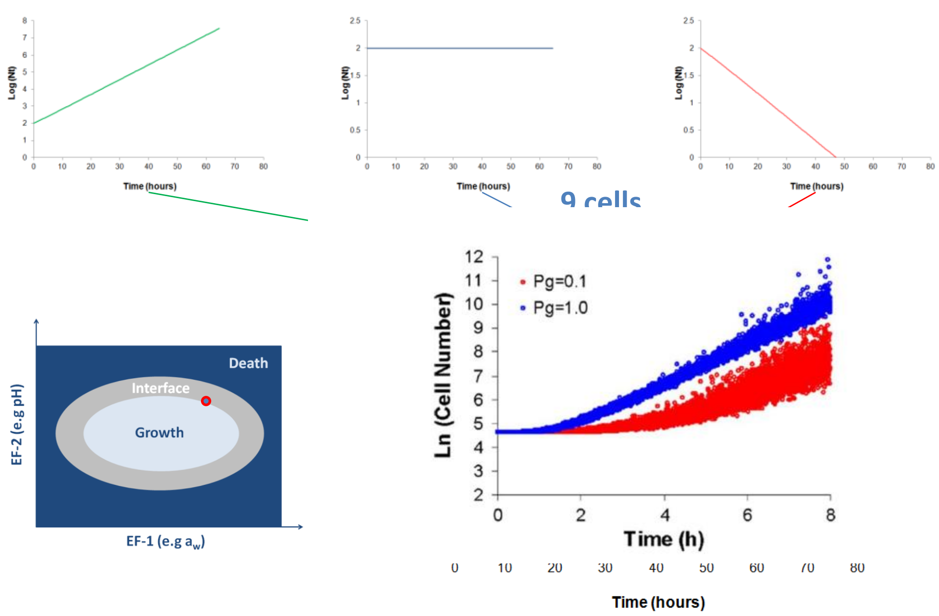


Interface

Population of 10 cells



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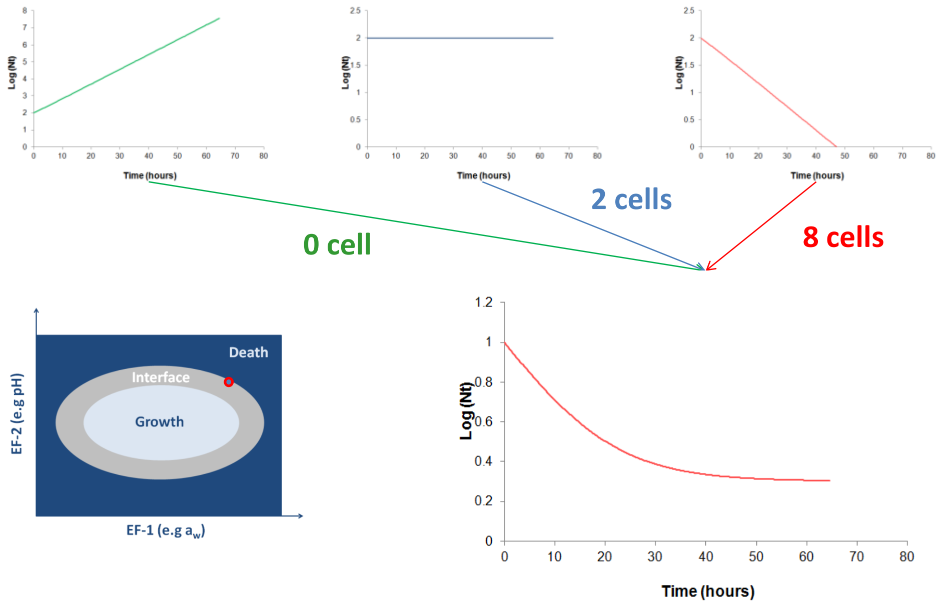
Population of 10 cells



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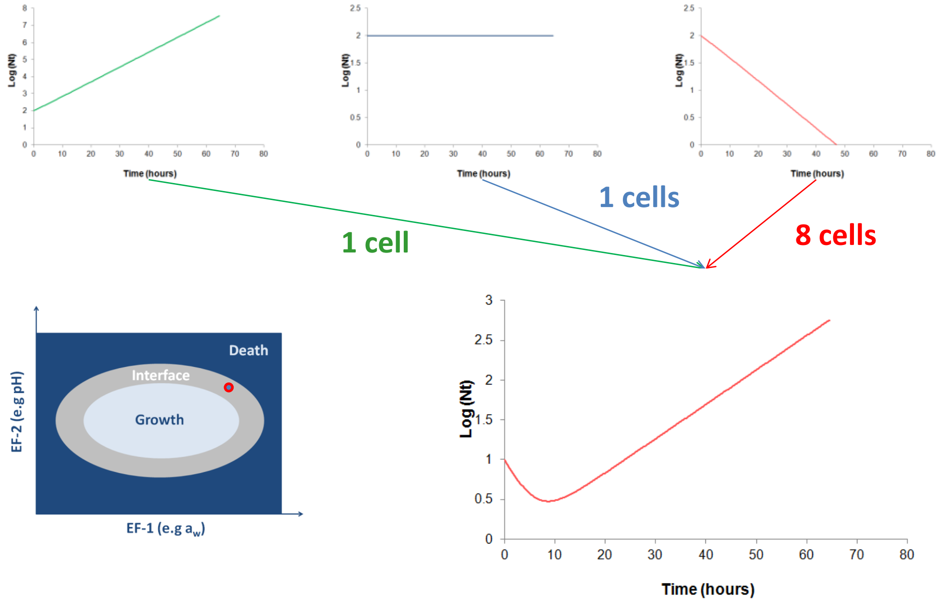
Population of 10 cells



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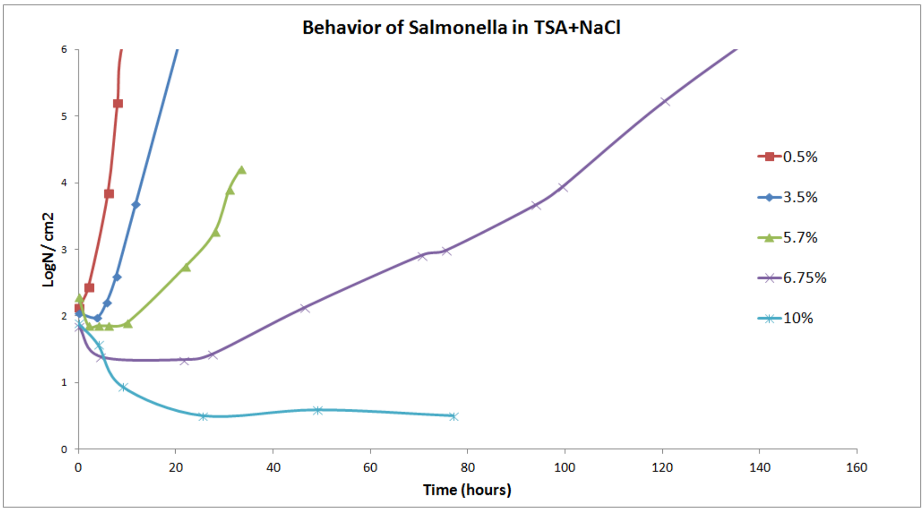

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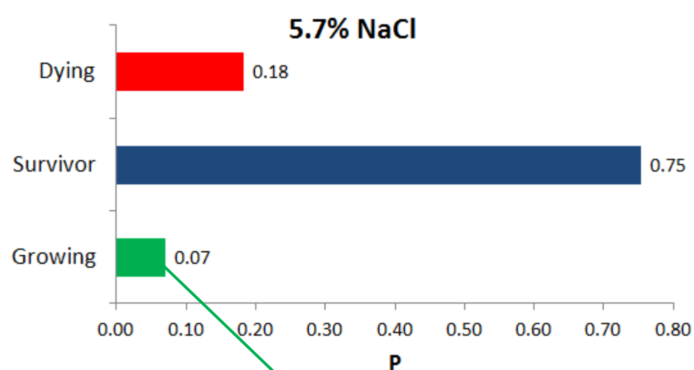
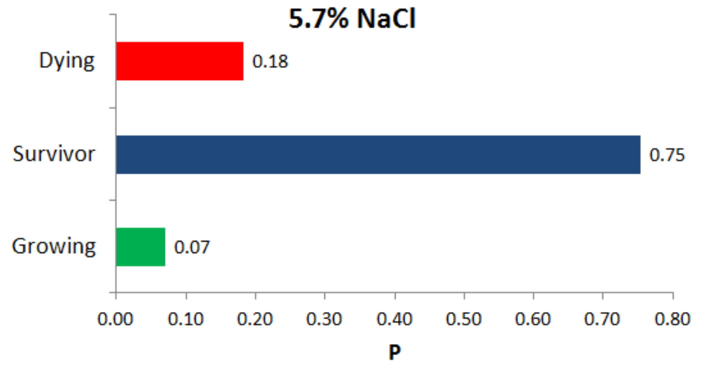
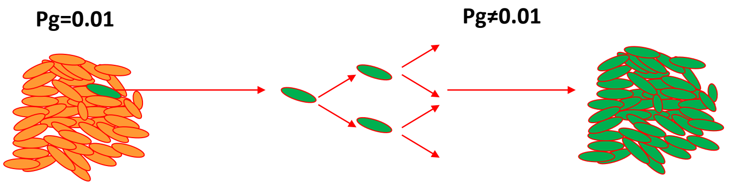


Do "daughter" cells follow the same behavior as the "mother" population

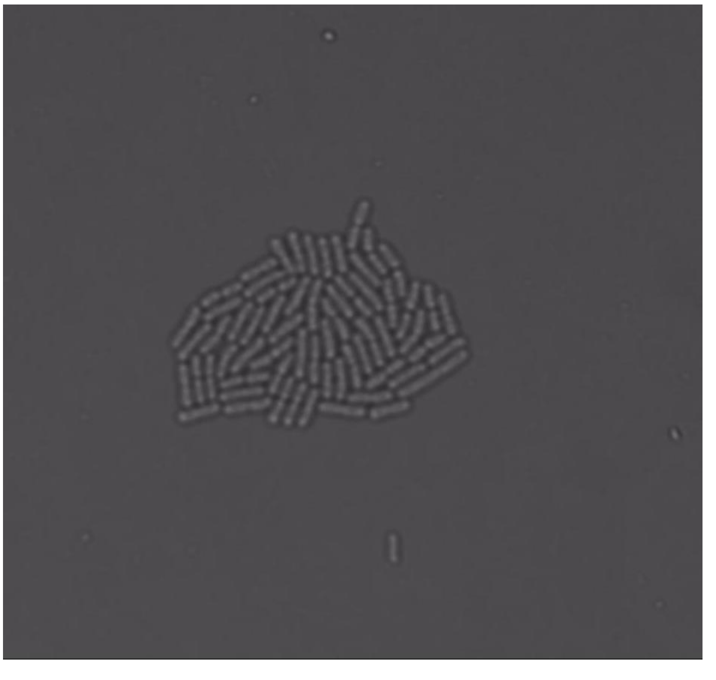
Interface





Interface

Salmonella exposed to 5.1% w/w NaCl and PI



Phenotypic heterogeneity (Noise)

➢The source of phenotypic heterogeneity is the Molecular noise: Differences in the production of a specific protein in genetically identical cells not related to genotype

Fundamental Questions-1

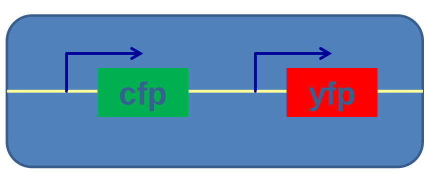
What is the source of molecular noise?

How can we tell if cell function is deterministic or stochastic?

Is noise inherent or a result of other factors such as differences in microenvironment?

The "Elowitz experiment",Nature, 2002

Two identical genes in the same cell tagged with different color (green and red Fluorescent Protein)



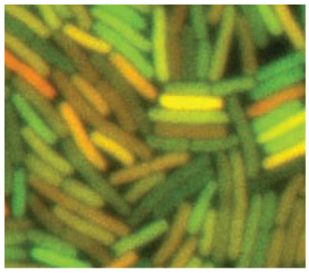
Is cell function deterministic or stochastic?

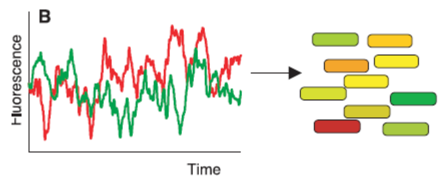
Stochastic Gene Expression in a Single Cell

Michael B. Elowitz,[1,2*] Arnold J. Levine,[1] Eric D. Siggia,[2] Peter S. Swain[2]

Michael Elowitz

The “Elowitz experiment”, Nature, 2002



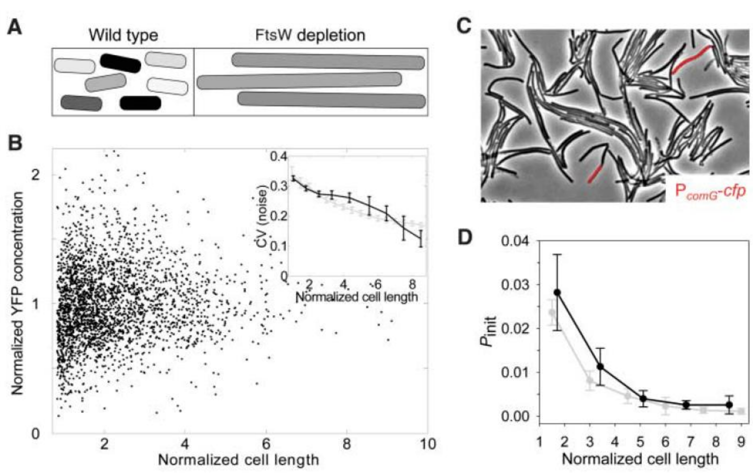


➢The source of molecular heterogeneity is the ***Noise in gene expression***:

The production of a specific protein in genetically identical cells in an essentially identical environment can differ among cells owing to *stochastic fluctuations (or noise)* during transcription and translation,

These differences *are epigenetic* and are typically not inherited by the variant’s progeny

Cells function is stochastic



***Noise decreased with cell length because of the higher number of molucels**

Suel et al., Nature, 2007

Phenotypic heterogeneity (Noise)

- The source of phenotypic heterogeneity is the Molecular noise: Differences in the production of a specific protein in genetically identical cells not related to genotype

Fundamental Questions-2

What is the role of noise?

Is Noise more than nuisance?

What is noise doing for (bacterial) life

"Order from Noise" Principle

Heinz Von Foerster, Phycicist-Cybernetician

On Self-Organizing Systems
and Their Environments*

H. VON FOERSTER
Department of Electrical Engineering, University of Illinois, Urbana, Illinois

"Noise plays a very important role as a trigger for the emergence of order in what is called self-organization or autopoiesis of a system"

Different from Schrodinger "Order from Disorder" (statistical noise)

Order from Noise Principle

Application: bird flocking example

Complexity from Noise

Henri Atlan,

Medical doctor, physicist and philosopher.

He applied the information theory in biology…

“..when assimilated, noise allows for a more complex order to emerge that can increase the system‘s adaptive capacities”..

Functional Role of Noise in bacteria

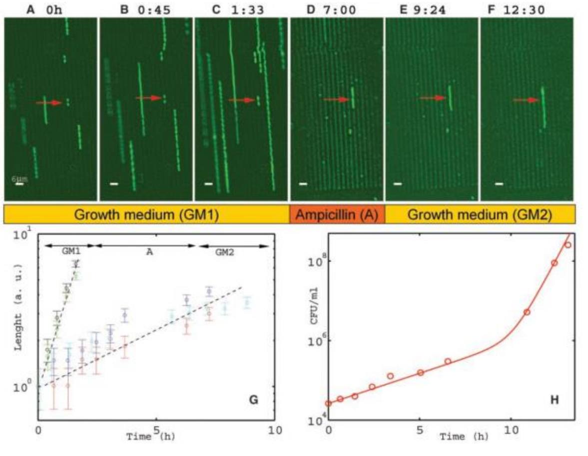
“Adaptive noise”

Examples

Bet-hetging: the production of offspring with variable phenotypes ensuring that at least one offspring will be appropriate (fit) under a given stress situation

Bistability (multi-stability): a gene regulatory network potentially which exhibits two (or more) discrete levels of gene expression (a high state and a low state) resulting in the existence of sub-populations

Example: Persistence to antibiotics



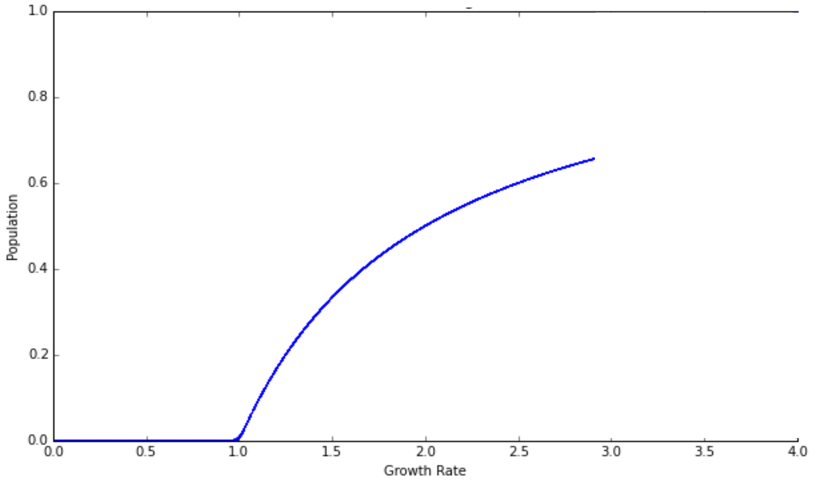
***Persistence is linked to preexisting heterogeneity in bacterial populations**

***Phenotypic switching occurrs between normally growing cells and persister cells having reduced growth rates.**

Balaban et. al., Science, 2004

Bistability (bifurcation) of the logistic equation

$$x_{n+1} = rx_n(1 - x_n)$$



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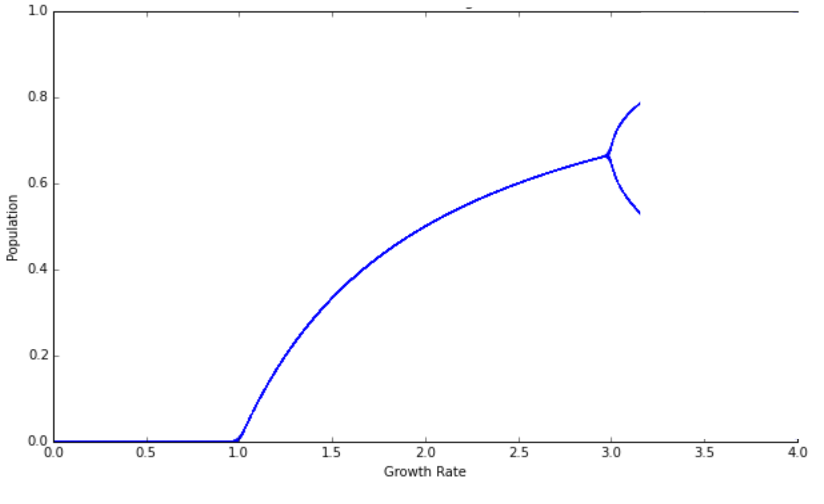
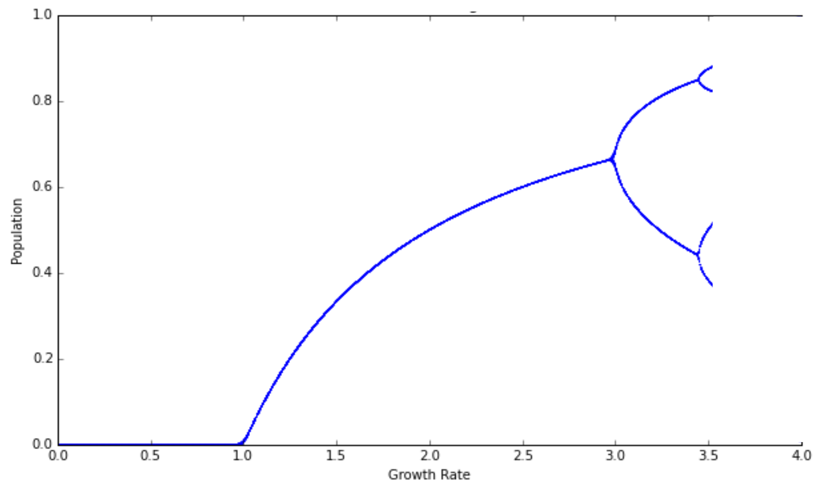


Bistability (bifurcation) of the logistic equation

$$x_{n+1} = r x_n (1 - x_n)$$



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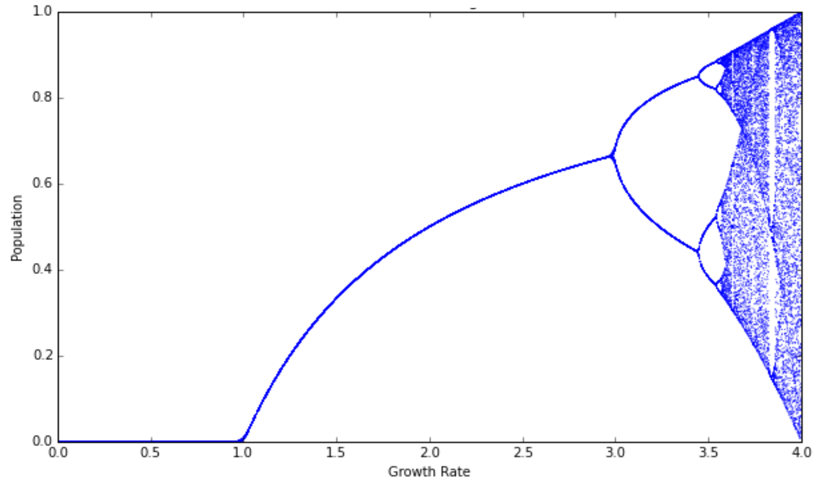


Challenges in single cell Food Microbiology

Top-down approach: monitoring phenotype

….high performance in predicting growth/inactivation rates at optimal and suboptimal environmental conditions…..

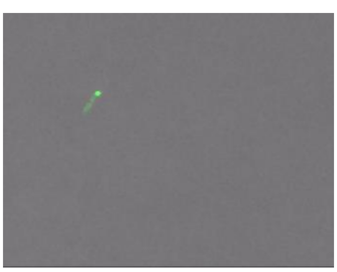
…. but when it comes to more complex questions and conditions (i.e. lag, physiological state, adaptative responses, conditions close to growth boundaries, etc) is generally not valid

Bottom-up approach: at molecular level

Challenges in single cell Food Microbiology

To exploit technological developments allow the collection of information and data on gene expression, protein and metabolic function even at the single cell level

Salmonella growth

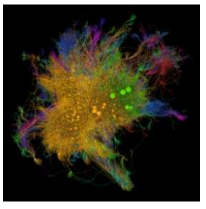


Salmonella Inactivation-PI
(cells exposed to food-related stress)

Challenges in single cell Food Microbiology

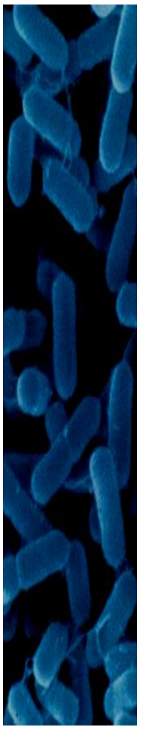
Bottom-up approach: at molecular level

From Reductionism…….to Complexity



New technologies
Scientists from other fields
New modeling tools

“Το όλον είναι ανώτερο από το άθροισμα των μερών του”
(The whole is “bigger” than the sum of its parts)
Aristotle 384-322 BC



Individual cell heterogeneity in Predictive Food Microbiology: Challenges in predicting a “noisy” world

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THANK YOU