Historical overview of Food Safety

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## Food Safety

Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use



Which fields in Food Microbiology contribute significant to Food Safety

World Health Day was celebrated on 7 April 2015, with WHO highlighting the challenges and opportunities associated with food safety under the slogan

'From farm to plate, make food safe'



Over 200 diseases transmitted through food and water containing harmful bacteria, parasites, viruses, chemical substances, result in (estimated) two million deaths every



## Microbiology started with:



- Antonie van Leeuwenhoek
- animalcules (small animals)
- first microbiologist
- never wrote a book
- letters to the Royal Society in London
- draper shop; interest in
  lens making (hobby)

## One of his microscopes ...



He investigated as much as possible: muscle fibers, spermatozoa, algae, blood flow, pepper ....



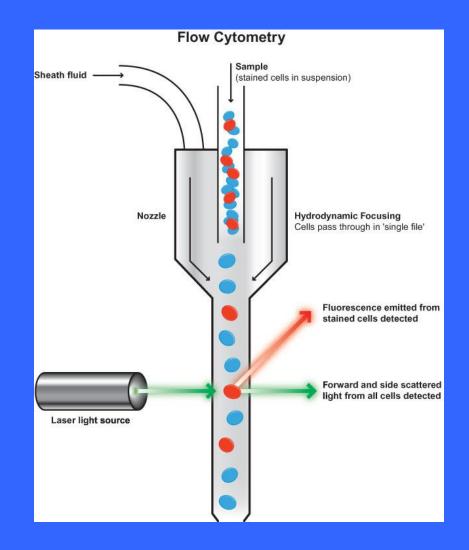
fig: A -.....D fig: B 5 ofig: G. fig: E: fig: r

## Fields in microbiology

 Methods to detect and identify microorganisms - (spoilage organisms and foodborne pathogens) microscopy, plating, DNA-based methods ... Ecophysiology of mo in foods/environment growth, survival, inactivation, stress response - Functional mo fermentation, probiotics - Risk analysis and modelling risk assessment - Hygiene in the production chain GMP, HACCP

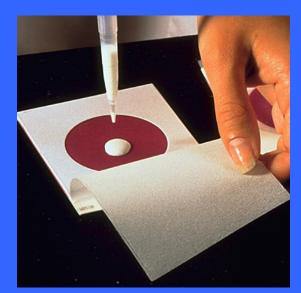
#### Microscopy

- AvL microscope,
- staining of microorganisms
- phase contrast microscopy
- DEFT
- electron microscopy
- Flowcytometer
- Bactoscan (raw milk) (up to 200 samples/h)



#### Growing microorganisms

- petri dishes
- petrifilm
- spiral plater







#### automated plating

#### Growing microorganisms

- (micro)anaerobic incubation







#### Growing microorganisms

- nutrient media

Bloedserum uit rundbloed, aseptisch verkregen, goed geschikt voor diphterie.

Gal, goed geschikt voor typhus- en colibacteriën.

Urine, pevat tot 3 % carbamide.

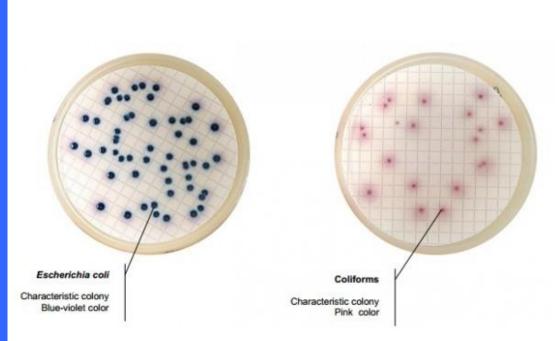
Carbanideoplossing (Söhngen 1909) voor ureumbacteriën : 30 ureum,  $0.5 \text{ K}_2 \text{HPO}_4$ , 10 calciumcitraat  $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_7)_2.4\text{H}_2\text{O}$ , 1000 leidingswater ; pH op 7.2.

Aardappel en brood met vleeschnat of met melk vochtig gemaakt : steriliseeren op 120° C.

Oplossing Pasteur :  $2 \text{ K}_2\text{HPO}_4$ ,  $0.2 \text{ Ca}_3(\text{PO}_4)_2$ ,  $0.2 \text{ MgSO}_4$ . 7H<sub>2</sub>O, 10 ammoniumtartraat, 100 suiker, 1000 water ; driemaal op 100° C. verwarmen.

#### Growing microorganisms

- intelligent media
- chromogenic media
- testing of culture media, ISO 11133
- Immunology
- ATP, TEMPO
- PCR, qPCR
- Impedance
   (Bactometer, Malthus)



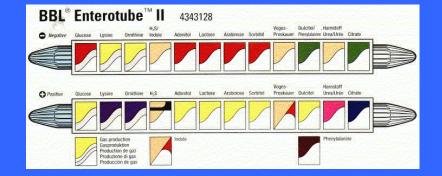
## Methods to identify microorganisms



#### **BD-Crystal**



#### numbers of tubes



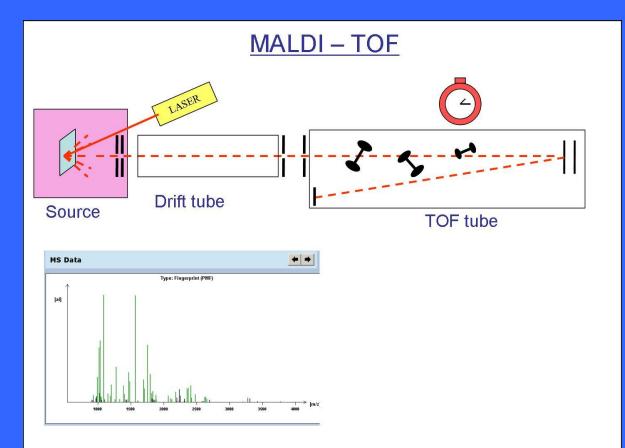
#### Enterotube



## Methods to identify microorganisms

#### Other methods

- immunology
- sequencing
- Malditof



## Shakespeare: the seven ages of man

# "ALL THE WORLD'S A STAGE, AND ALL THE MEN AND WOMEN MERELY PLAYERS: THEY HAVE THEIR EXITS AND THEIR ENTRANCES; AND ONE MAN IN HIS TIME PLAYS MANY PARTS, HIS ACTS BEING SEVEN AGES."

WILLIAM SHAKESPEARE

🕲 Lifehack Quotes

#### Sculpture

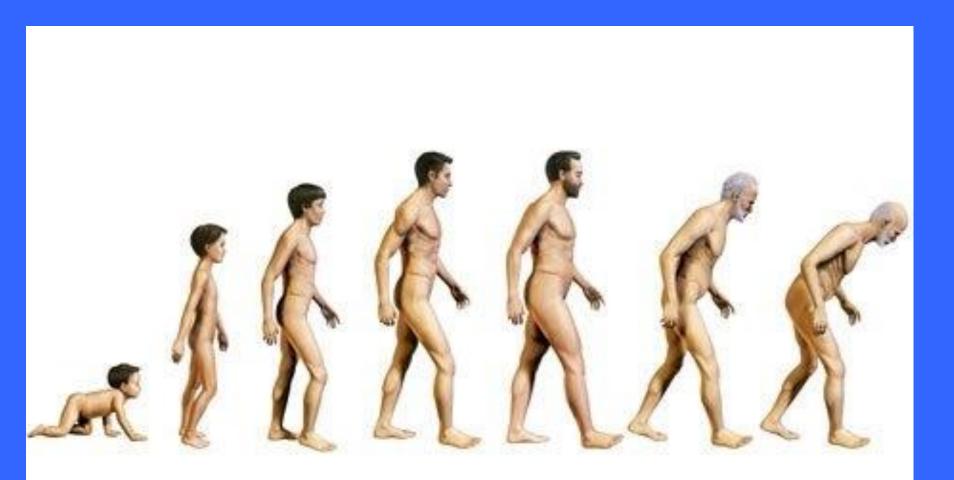
#### seven ages of man

#### London

by Lonpicman



#### more or less the same ...





## The seven ages of man ...

- infancy: helpless baby, knows little
- whining schoolboy: goes to school, not confident yet
- the lover: sentimental, trying to express his feelings
- the soldier: hot headed, making reputation
- the justice: acquired wisdom
- old age: loses his firmness and assertiveness
- incapacity: dependent on others, unable to interact

## Looking back to microscopy ...

infancy, whining schoolboy, the lover, the soldier all fields: AvL, phase contrast, DEFT, Bactoscan, FC, EM

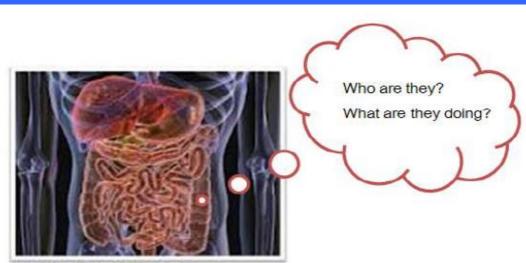
the justice phase contrast, Bactoscan, FC, EM

old age DEFT

incapacity DEFT

As soon as growth of mo was possible, a lot of questions arose:

- why is this mo there?
- which factors involved?
- how to investigate?
- possible to change factors to control the growth of mo?



Migdimon geties countries gedowitz jguts. 8%

### it started with intrinsic factors

- structure
- water activity
- pH
- preservatives
- etcetera



#### extrinsic factors

- modified atmosphere packaging
- relative humidity
- storage temperature
- process factors
- temperature
- ultra high pressure
- pulsed electric fields

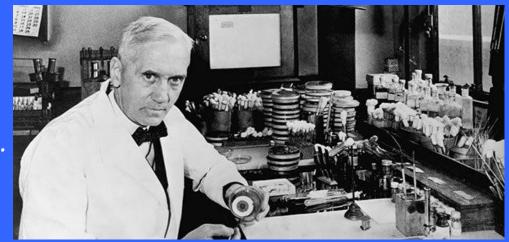




#### implicit factors

- synergism ... working together starch ... amylase ... sugars ... growth other mo yoghurt fermentation
- antagonism ... preventing growth of other mo Penicillium ... antibiotics ... Fleming





possibilities for preservation ... (antibiotics '60-'70)!

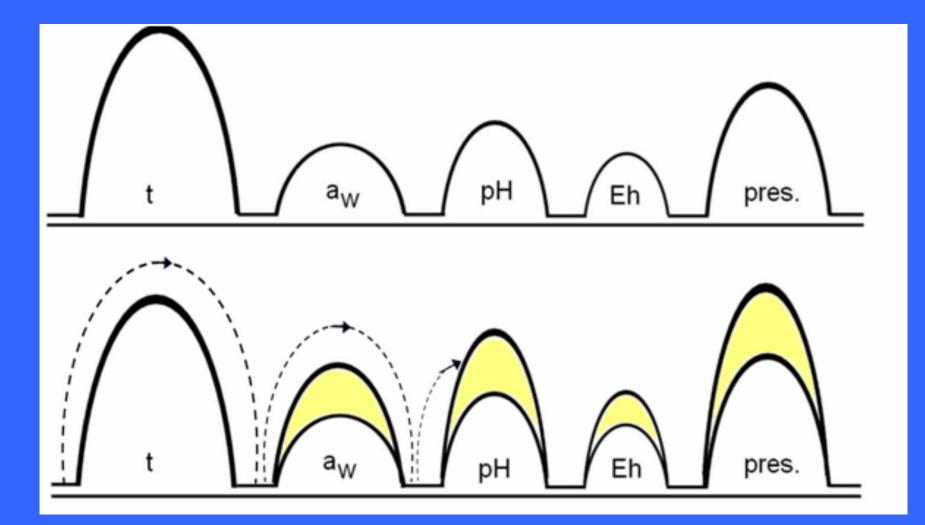
With the growing knowledge preservation of food products was possible

However, some researchers were too enthousiastic i.e. bacteriocines, extracts from herbs



soldier, stage 4

## Ecophysiology of mo: combining factors = hurdle technology (Leistner 2000), wisdom



#### Fermentation

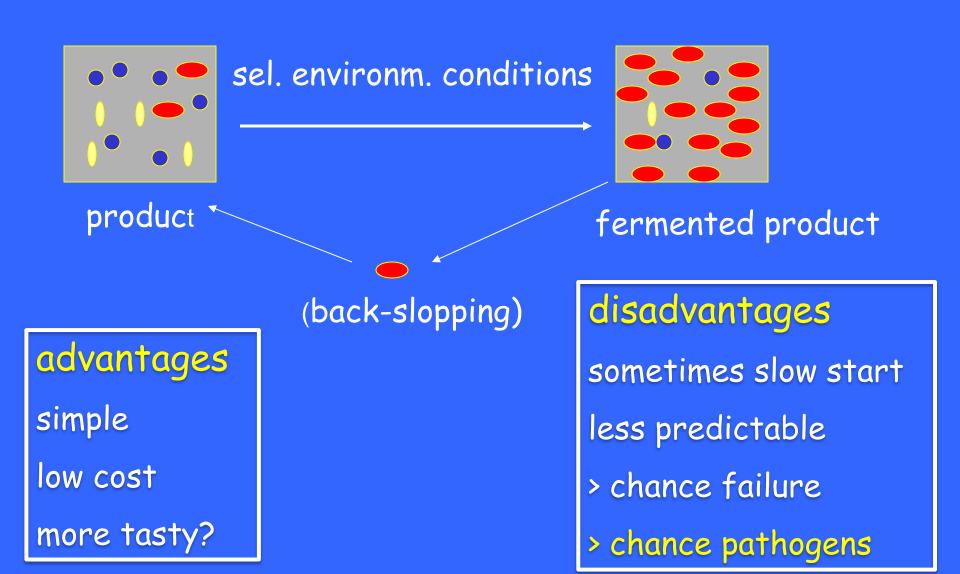
Fermentation: The chemical breakdown of a substance by bacteria, yeasts, or other mo (or its enzymes)
just a small step from the previous slide

- some people call an unknown fermented product spoiled

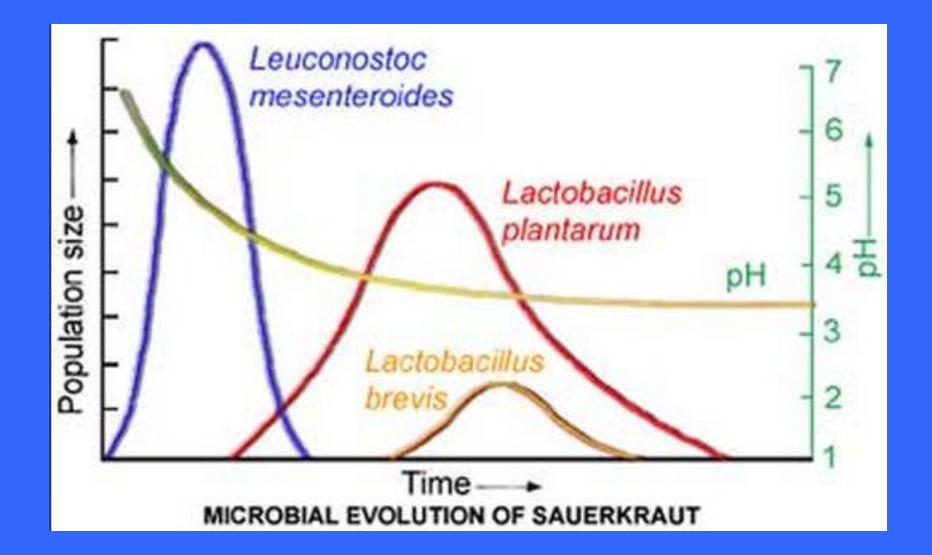




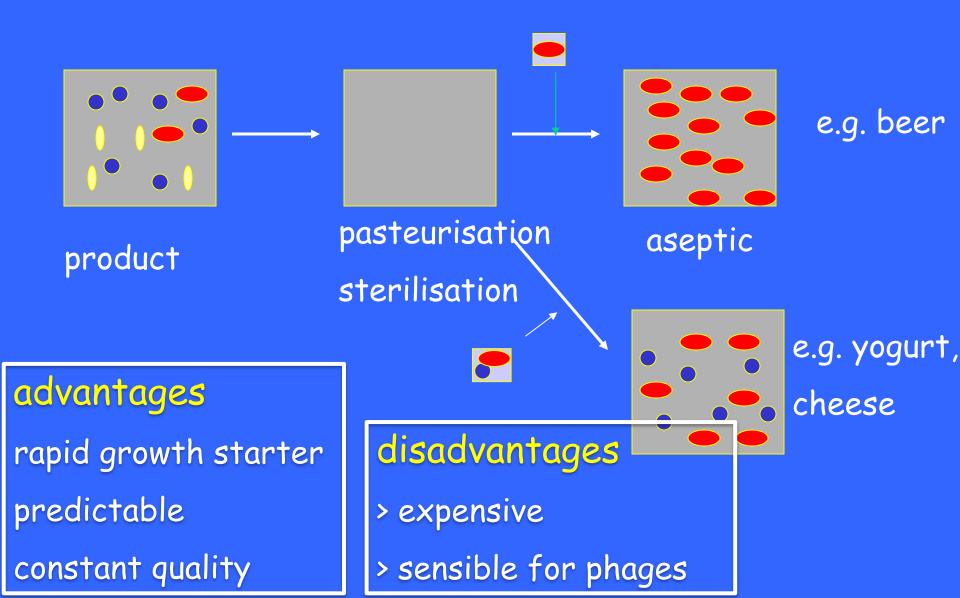
#### Natural fermentation



### Fermentation



#### Fermentation with selected starters



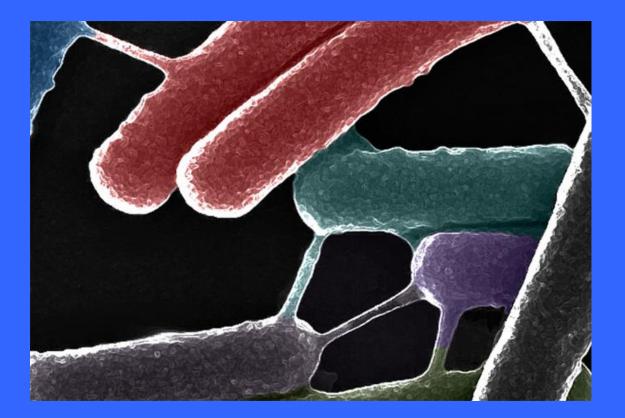
## **Probiotics:** definition

- Living microbial food supplement,
   stimulating health by improving microbial balance
   human intestine
- Minimal >10<sup>8</sup> 10<sup>9</sup> cfu necessary
- Addition to normal diet
- Active in small intestine (competition)

Stimulation immune system is a rather vague claim

#### Probiotics

Some scientists call it 'magic bacteria',

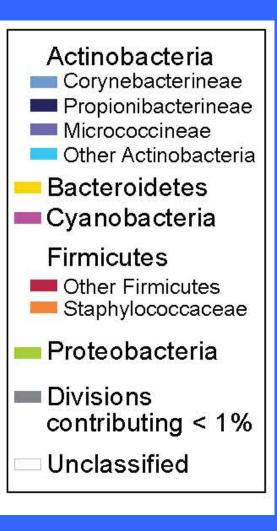


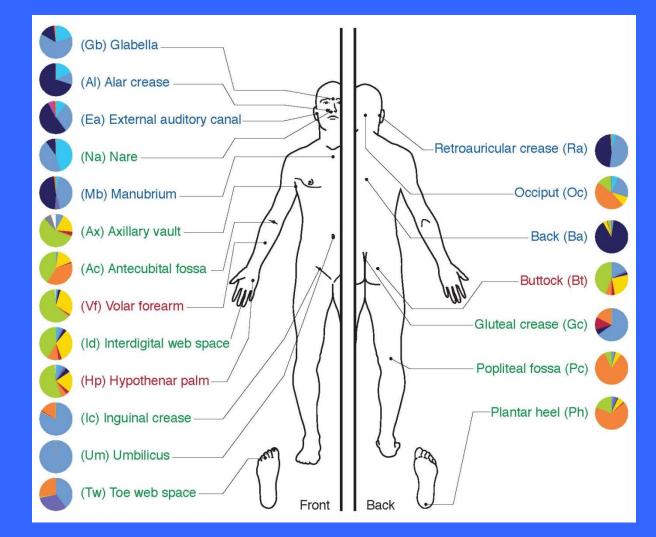
#### At Danone and Yakult the cash registers jingle

#### Fermentation and probiotics

- Natural fermentation in the first three stages of age: infancy, schoolboy, lover
  - Fermentation with selected starters acquired wisdom
- Probiotics still in the stages infancy soldier

## Not fermentation .... but microbiota accumulation of mo





#### A microbiota is 'the ecological community of commensal, symbiotic and pathogenic mo that literally share our body space'.

Joshua Lederberg coined the term, emphasizing the importance of mo inhabiting the human body in health and disease.



## Microbiotica

Many researchers think the human microbiome has potentially overwhelming impact on human health

It may have a role in auto-immune diseases like diabetes, rheumatoid arthritis, muscular dystrophy, multiple sclerosos, fibriomyalgia, and perhaps some cancers.

Furthermore, obesity, schizophrenia, depression, and other neuro-chemical imbalances.

Be careful: Researchers always say: it may also ...

Still many speculations: stage 4, the soldier

#### Back to the ecophysiology of mo

The group of mo consist of:



useful

#### annoying, and

#### dangerous mo



#### The Bible for food microbiologists Han Joosten, WU<u>R ex Nestlé</u>

Micro-Facts: Peter Wareing, Felicity Stuart and Rhea Fernandes, 7<sup>th</sup> ed. 2010

The working companion for food microbiologists



## Some foodborne pathogens

1880 1892 1897 Staphylococcus aureus Clostridium botulinum Cl. perfringens

1900 *Salmonella,* named in honour of DE Salmon

'-50 1977 1980 1982 '-80

Bacillus cereus, Vibrio parahaemolyticus Campylobacter Cronobacter STEC Listeria monocytogenes

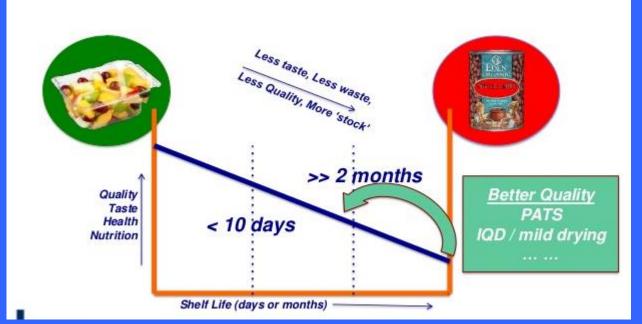
**AB-resistent mo** 

# Food Safety: control of pathogens in food products

With the hurdles: Aw, pH, preservatives, low or high temperature, UHP, PEF, irradiation, (natural) antimicrobial systems ...

Some of the methods are quite effective

Demand for mild preservation



## Survival strategies for pathogens

Microbiological stress respons and minimal processing

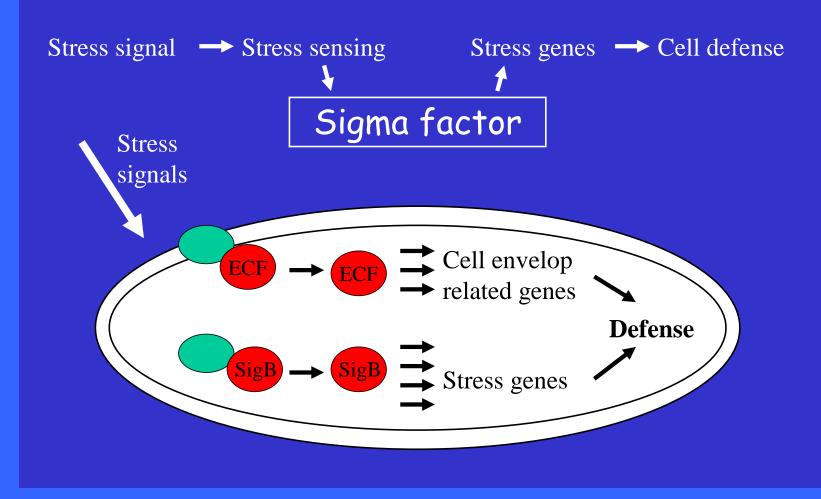
Consumers ask for mild processed products

New methods for control food safety: UHP, PEF, ultrasound, combinations of preservatives, bacteriocins, essential oils ...

Stress response: acquired wisdom, stage 5

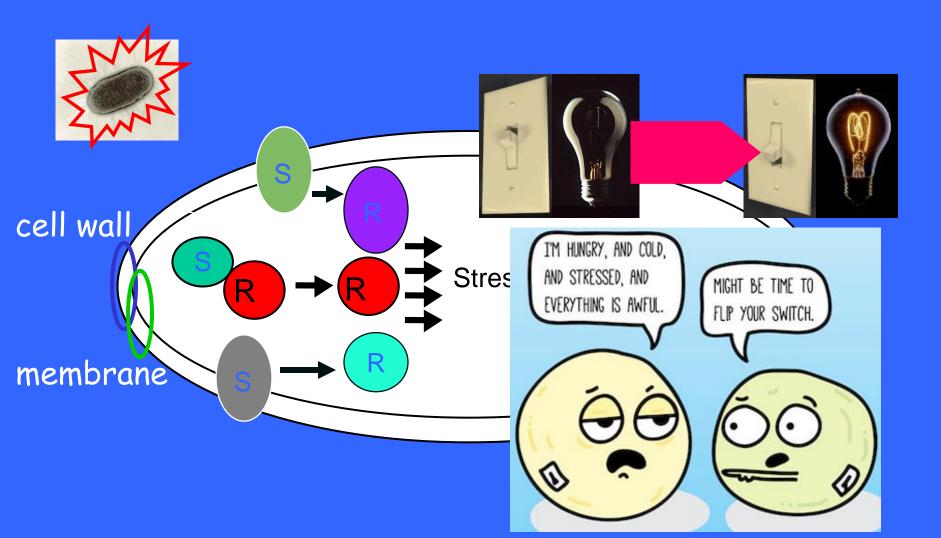
## Important factors for surviving stress

Central role for sigma factors ( $\sigma^{B}$  and ECF) in stress response

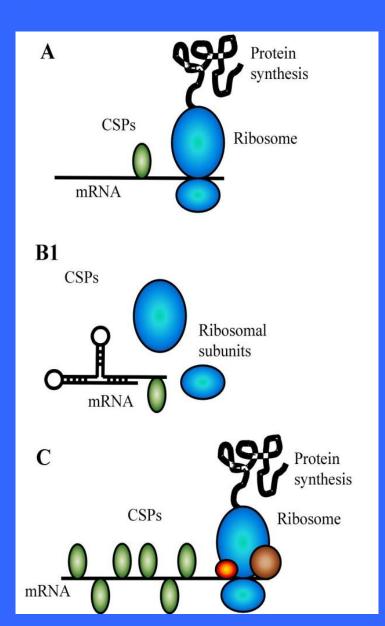


#### Activation stress response

Activ. sensors > activ. regulators > activ. genes > activ. defense



## Cold shock proteins in survival of stress



## Stress-induced expression of Sigma factor B

sigB mRNA concentrations increase during stress

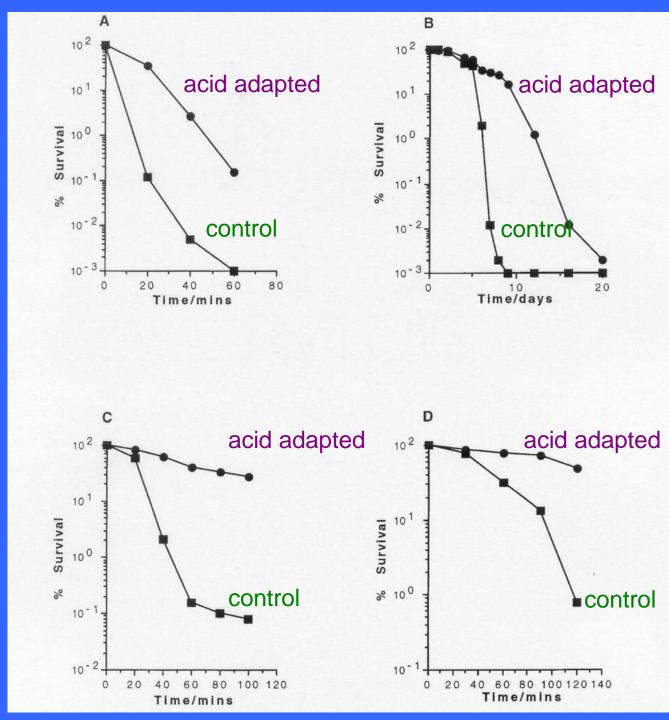


1. mid-exp. phase 2.4% NaCl 3.2% ethanol 4.1 mM EDTA 5. 0.15% H<sub>2</sub>O<sub>2</sub> 6. pH 5.3 7.48°C 8.4°C 9. stat. phase

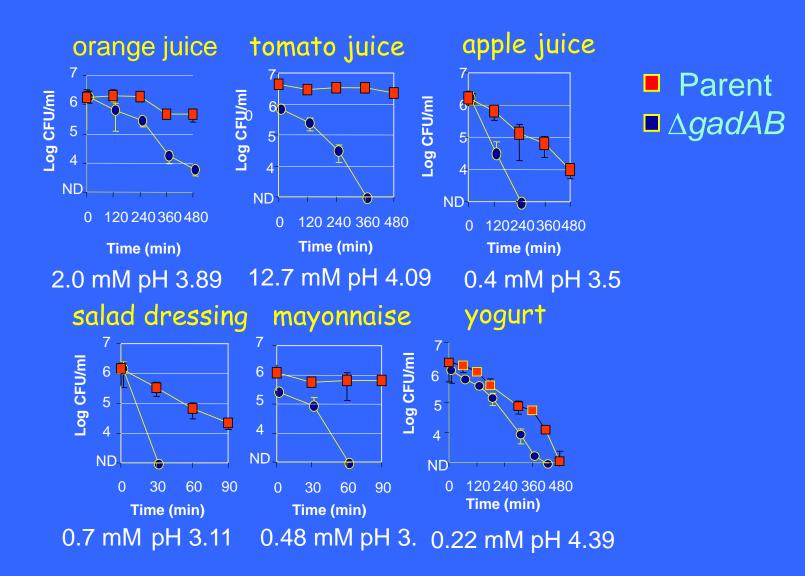
Becker et al., 1998. J. Bacteriol. 180, 4547

Better survival of acid-adapted *Lm* in comparison with control strains

exposure to: A, Heat (52° C) B, NaCl (2.5 M) C,  $H_2O_2$ D, Ethanol (15%)

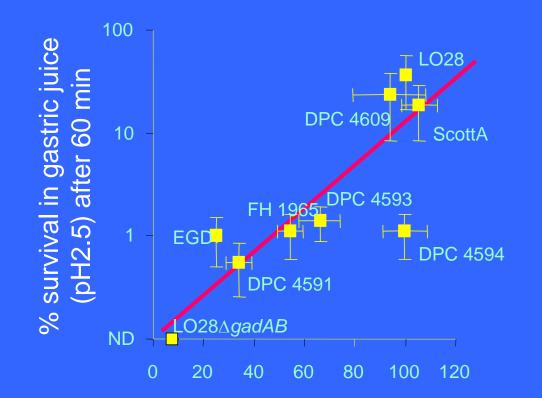


# *Lm* survival in acid products role glutaminate decarboxylase (GadAB)



## Better survival for *Lm* in gastric juice?

Plotted: GAD activity vs. survival in gastric juice



no strain was more resistant than could be expected on the grounds of GAD activity

%GAD activity with respect to LO28

glutaminate decarboxylase system important low pH survival

## Developments in Food Microbiology

- Microscopy: light, phase contrast, electron, FC

- Ecophysiology: growth, survival, inactivation, stress response

- Functional mo and probiotics

- Pathogens: DR, foodborne diseases, prevalence

- Risk analysis

# **STRUCTURE OF RISK ANALYSIS**

#### **Risk Assessment**

- Hazard Identification
- Hazard Characterization
- Exposure Assessment <sup>\*</sup>
- Risk Characterization

#### **Risk Management**

- Risk Evaluation
- Option Assessment
- Option Implementation
  - Monitoring & Review

#### **Risk Communication**

### Risk assessment: how to do?

A double fresh meal, with raw and semi-cooked ingredients mixed and MAP packaged, called TV-dinner in the USA

shelf life from 1-2 week heating in microwave by the consumer



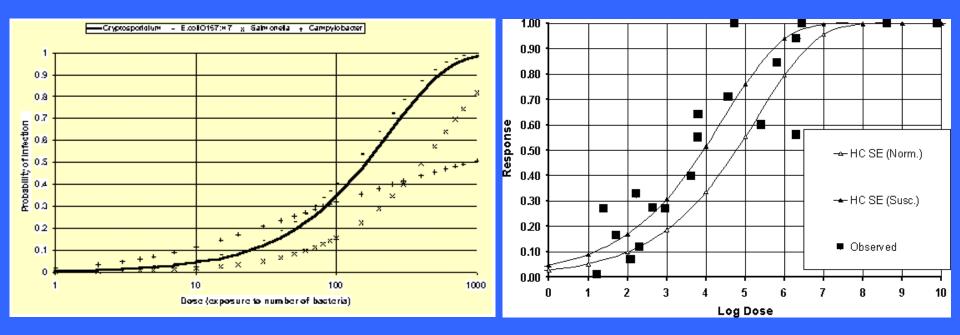
which foodborne pathogens should be inactivated?

# Hoe great is the risk of mortality (NL)

risk of mortality	N/10 <sup>6</sup>	cause of death
1: 115	8.800	total # deaths
1: 10.000	100	infectious diseases
1: 10.000	100	suicide
1: 15.000	75	traffic
1: 55.000	16	food
1: 200.000	6	drowning
1:20.000.000	0.05	lightning

# Important things to know of pathogens (1)

food borne infection or – intoxication; incidence; DR; incubation time; symptoms; duration; dead products involved; sources; environmental cycle; ecophysiology; control in food ... stage 5



#### How to select relevant foodborne pathogens



# List them all Thereafter, use Risk Ranger to select the 3 most important



Listed bacterial foodborne pathogens							
Pathogen	Meat	Fish	Vegetables Pas	sta/Rice			
Aeromonas	=	=	=				
B. cereus	=	=	=	=			
C. botulinum	=	=	=				
C. perfringens	=	=	=				
Campylobacter	=	=	=				
E. coli	=	=	=	?			
L. monocytogen	nes =	=	=	=			
Salmonella	=	=	=	?			
S. aureus	=	=	=	=			
Vibrio	=	=	=				

## Risk Ranger to identify most important ones

download from:

http://www.foodsafetycentre.com.au/docs/RiskRanger.xls

- answers on 11 questions, result in a 'risk ranking'
- differences expressed on a log scale

just for your convenience, divided in a scale from 0-100

- mild:1: 10<sup>9</sup> people ill in 100 year
- severe: everybody dies after eating one meal
- 6 RR units: about a factor 10 difference

### Risk Ranger: example of a question

#### **B.** PROBABILITY OF EXPOSURE TO FOOD

#### **3 Frequency of Consumption**

daily	*
weekly	
monthly	
a few times per year	
OTHER	Ŧ

#### Risk Ranger: a result

## **RISK ESTIMATES**

1.42E-07

2.54E+02

46

probability of illness per day per consumer of interest (*Pinf x Pexp*)

total predicted illnesses/annum in population of interest

RISK RANKING (0 to 100)



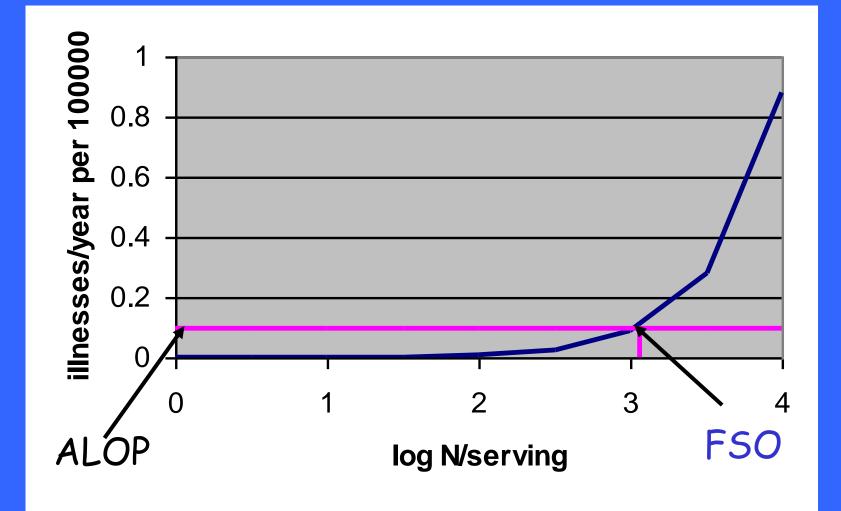
#### **FSO**

Food Safety Objective (cfu/g, or presence of a pathogen (%)

#### based on ALOP

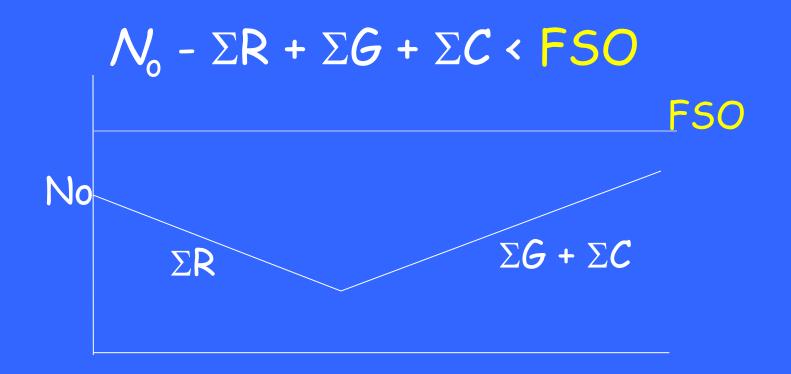
Appropriate Level Of Protection (# persons ill, in hospital, or death/year)





based on dose/response and # of portions

## FSO (definition by ICMSF)



Food Safety Objective, formulated by the government



## $N_{o} - \Sigma R + \Sigma G + \Sigma C < FSO$

Industry Microbiological research Quantitative microbiology Government DR, Epidemiology, Consumption



primary production  $N_0 - \Sigma R + \Sigma G + \Sigma C < PO_1$ 

performance objective

food industry N\_-ΣR+ΣG+ΣC<PO

PC: performance criterion (6D) consumer $N_0 - \Sigma R + \Sigma G + \Sigma C < FSO$ 

process/product criteria (71.5°C, 15s)

#### Reminder

ALOP

Appropriate Level of Protection ilness, and t/year; DR, consumption

FSO

PC

Food Safety Objective cfu/g or prevalence (%)

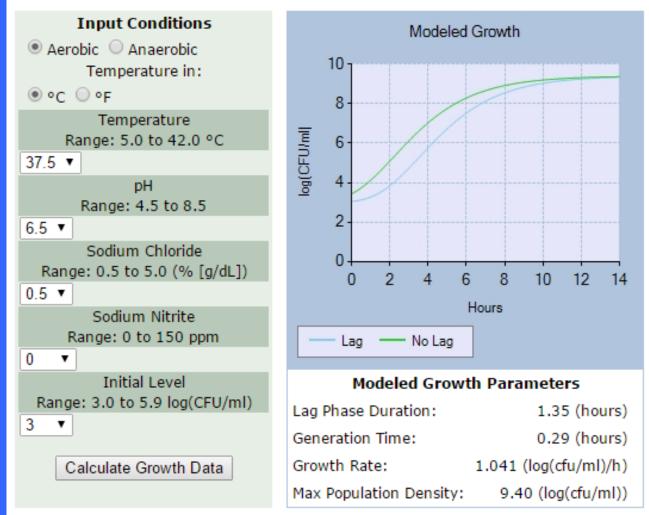
PO Performance Objective cfu/g or prevalence (%)

Performance Criterion
>6D inactivatie, <3D growth

Process criterion: 15s 71.5°C and Product criterion: pH<4.6

# Use a model to predict the growth of the three selected pathogens (Combase, PMP, or others, e.g. FSSP)

Growth Model: Escherichia coli [0157:H7] (Broth Culture, Aerobic)

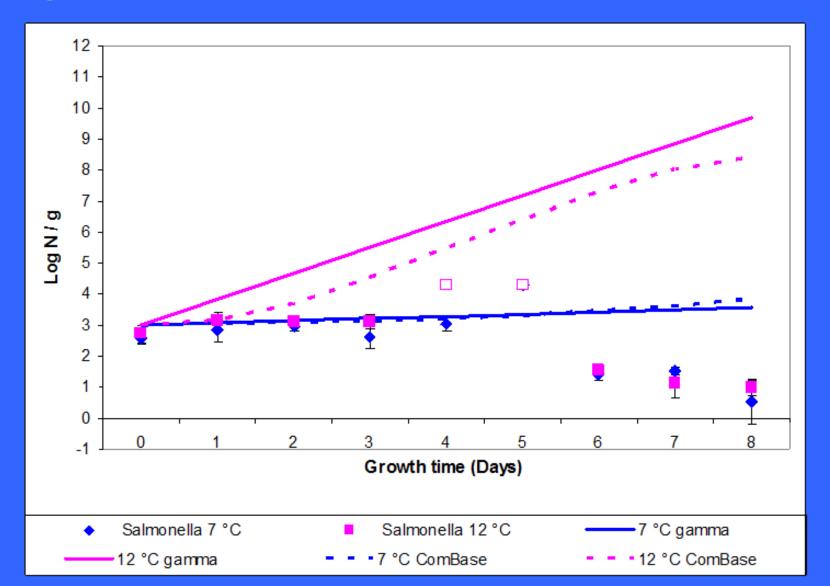


#### Thereafter, perform challenge tests

# A safe double fresh meal, you will get by combining the data obtained

foodborn pathogen	initial (LogN/g) raw materials	growth at retail and consumer	post process contam.	levels before heating (LogN/g)	inactivation microwave estimated	level at consumption (LogN/g)
Listeria	1	2	0	3	6	?
Salmonella	0	1	0	1	6	?
<i>Bacillus</i> vegetative	4	1	0	5	6	?

# Prediction and challenge test for the growth of *Salmonella* in a TV dinner



# Important things to know of mo in food

Control in food

- \* GMP, HACCP
- \* refrigeration, pasteurisation, sterilisation, etc.
- \* preservatives

and consumer education

# Control of food production

Prerequisite:

Basics/common sense

GMP, GHP, GLP

Training/long life learning

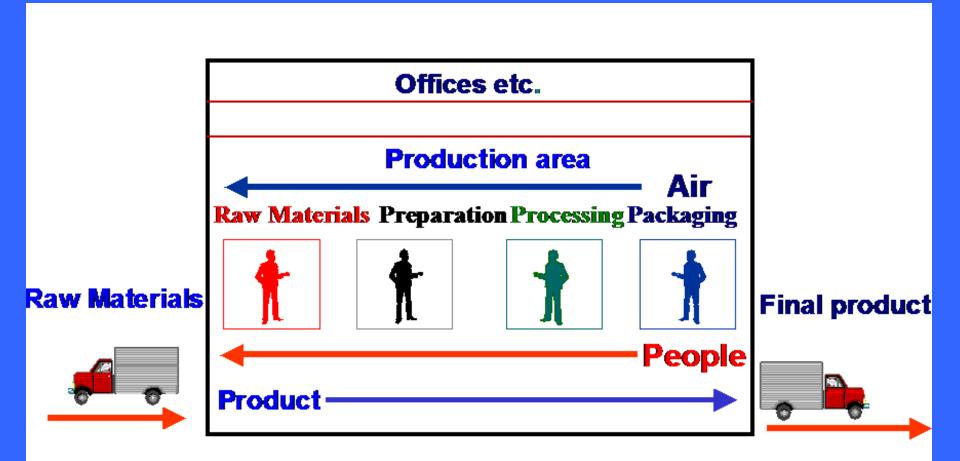
QRA

prevent stupid errors

Contributes to food safety, stage 5, wisdom

HACCP

# Zoning: prevention of (cross) contamination





#### between areas to prevent (cross) contamination

also for internal traffic,

production apparatus

product flow

tools





# Zoning: changing shoes?



## Zoning: corridor for visitors



## Sampling the environment

 When, what ... depends on type of factory know your basic level

\* product spill, - scrape, dust, etc.



## Sampling the environment

#### A: environment close to the product

#### B: surrounding areas

#### C: remote places

## Sampling the environment

Differentiation of samples

- A: surfaces in contact with the product
- B: surrounding areas
- C: remote areas

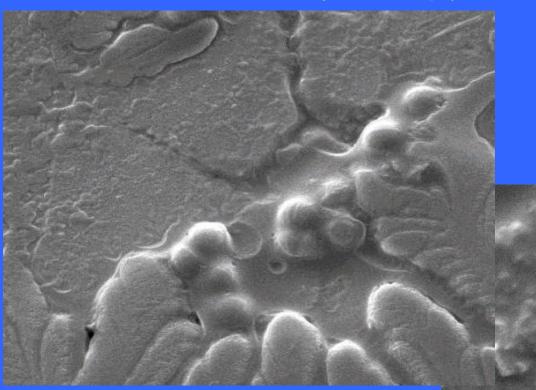
General hygiene: A 60%; B 40%

Pathogens: A 60%; B 30%; C 10%

## Transfer of mo using rodac plates

	Ν	Recovery (%)	
		1×	5x
B. cereus	50	18 ± 6	33 ± 11
S. aureus	10	46 ± 14	88 ± 26
<i>S</i> . Ent.	14	23 ± 6	42 ± 12
C. jejuni	10	7 ± 3	14 ± 7

# Transfer determined by morphology/size of mo?



Campylobacter

1 M m

Staph. aureus

## Transfer mo



#### contact time (10 sec)

pressure (500g)

device available at BioMérieux, VWR







TILBAKEKALLES: Hundrevis skal ha blitt syke etter å ha spist norsk røkelaks solgt i USA og Nederland. Saken har vakt oppmerksomhet verden over.

FRANK PERRY/SKJERMDUMP AFP/E24

## Gir gresk fabrikk skylden for salmonella i «norsk laks»





#### Conclusions for food producers

- \* Raw products, which are contaminated with pathogens will remain a hazard, even when the food is held at low temperatures (refrigerator or freezer)
- \* Heat treatment (temperature up to 70°C, or higher) will inactivate pathogens, usually not spores
- \* Most other methods only decrease mo in numbers
- Be aware of stress in mo
- Clean and disinfect thoroughly
- \* Be careful in case of a long shelf life

#### Conclusions for consumers

For the safety of foods, which enter kitchens as raw agricultural commodities, including meat, poultry, seafood and vegetables, one cannot rely solely on animal health programs and sanitation

A certain knowledge is necessary to prepare food as safe as possible for family and quests



