ADRIA DÉVELOPPEMENT
BRETAGNE OCCIDENTALE UNIVERSITY (LUBEM) IN QUIMPER
ACTIA

UMT SPORE-RISK
RISKS LINKED TO SPORE-FORMING BACTERIA

Sym'Previus for beginners

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ADRIA DEVELOPPEMENT

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Food Safety & Quality

- codex alimentarius is a collection of international standards, guidelines and codes of practices to ensure food quality & safety, protect consumer health and promote fair trade

- microbial risk assessment (MRA) is adopted by regulators globally to assess risks posed by pathogenic microorganisms in the food chain

- for ready-to-eat food which supports microbial growth, various guidelines are available to ensure microbiological quality & safety for specified food, process, storage and handling conditions

EC Regulation

✓ Regulation EC 2073/2005 - microbiological criteria in food -

« Food business operators shall take measures to ensure that the food safety criteria applicable throughout the shelf-life of the products, can be met under reasonably foreseeable conditions of distribution, storage and use. »

✓ Annex II regulation EC 2073/2005

« Food business operators shall conduct additional studies which may include:

- predictive mathematical modelling for given microorganism in food

- studies to investigate the ability of the micro-organism of concern to grow or survive in the product during shelf-life »

Training
Audit - Consulting

Research & Innovation

outline

rationale on challenge test & predictive microbiology

01 Identify bacterial hazards & assist product formulation

02 Validate food shelf-life

03 Quantify process efficiency

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Rationale

Challenge testing is one of the recognized approaches used to validate control measures within the HACCP system, as well as to assess microbiological safety and suitability

Available standards or guidance documents

- EC Regulation 2073/2005 on microbiological criteria for foodstuffs
- US NACMF Guidance for inoculated pack/challenge study protocols
- NF V01-009 Standard Guidelines for the implementation of microbiological challenge test for growth

- Document guide SANCO on *L. monocytogenes* shelf-life studies for RTE
- Health canada: *L. monocytogenes* challenge testing of refrigerated RTE

Rationale

Predictive mathematical modeling is one of the recognized approaches used to assess microbiological safety and suitability

The basic idea underlying predictive microbiology is that the behavior of microorganisms is deterministic and able to be predicted from knowledge of :

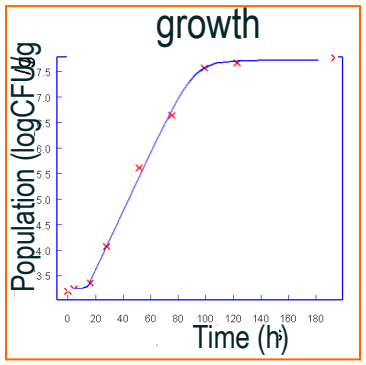
- microorganism itself (strain)
- its immediate environment (food, process/storage)

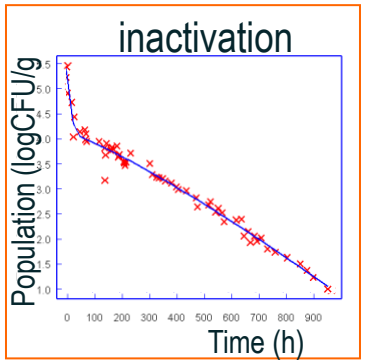
Rationale

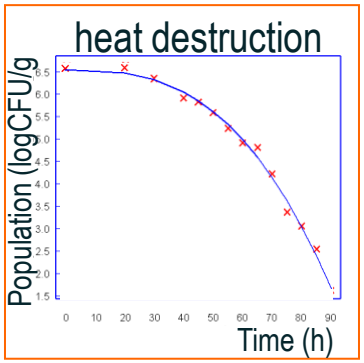
Predictive mathematical modeling is one of the recognized approaches used to assess microbiological safety and suitability

what: Qualitative & quantitative estimation of microbial behaviour as a function of environmental conditions (pH, a_w, temperature, [active compound] …)

how: Use of mathematical models to simulate growth, survival, inactivation of micro-organisms taking into account specific parameters with +/- biological significance





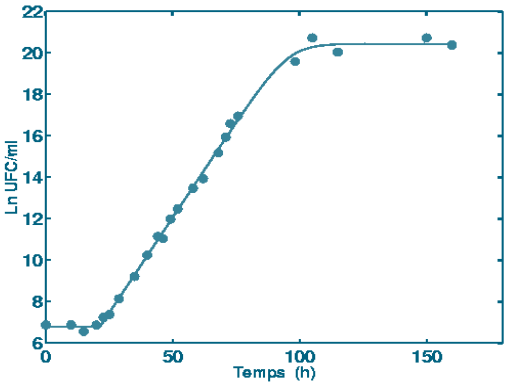


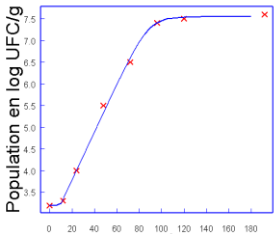
Rationale

Predictive mathematical modeling describes & quantifies the evolution of microbial population taking into account intrinsic (pH, aw, organic acid …) and extrinsic characteristics (MAP, storage …)

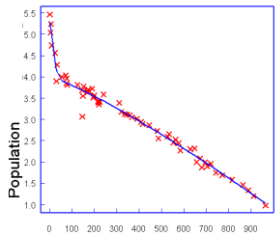
Primary models

Evolution of microbial population as a function of time for given conditions. Ex: microbial growth (μ_{max}), kill curves or inactivation kinetics (D, δ)

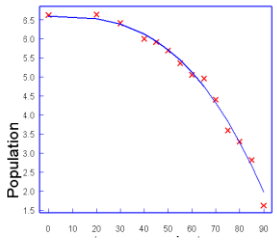




Growth rate: μ_{max}
Lag phase: lag
Population: $\log N_0$
Population: $\log N_{max}$



Pop reduction: δ
Shape : p
State: α
Population: $\log N_0$



Pop reduction: D or δ
Shape : p
Population: $\log N_0$

Rationale

Predictive mathematical modeling describes & quantifies the evolution of microbial population taking into account intrinsic (pH, aw, organic acid …) and extrinsic characteristics (MAP, storage …)

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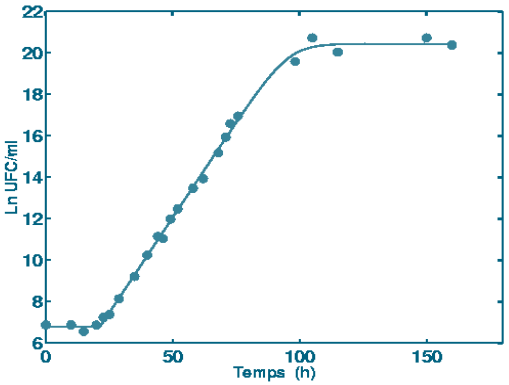
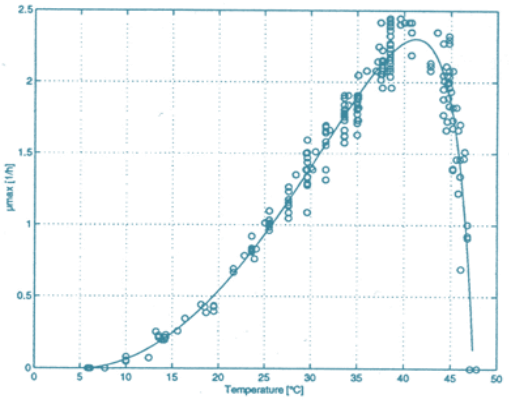


Secondary models

Impact of environmental conditions on primary models parameters (growth rate or population reduction)

> Polynomial models and modular models



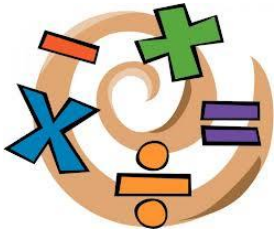
Rationale

Predictive mathematical modeling describes & quantifies the evolution of microbial population taking into account intrinsic (pH, aw, organic acid …) and extrinsic characteristics (MAP, storage …)

Tertiary models

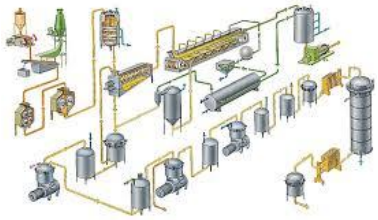
Decision making tool to simulate microbial behavious and test several scenarii of industrial interest

microbiology



mathematics

statistics

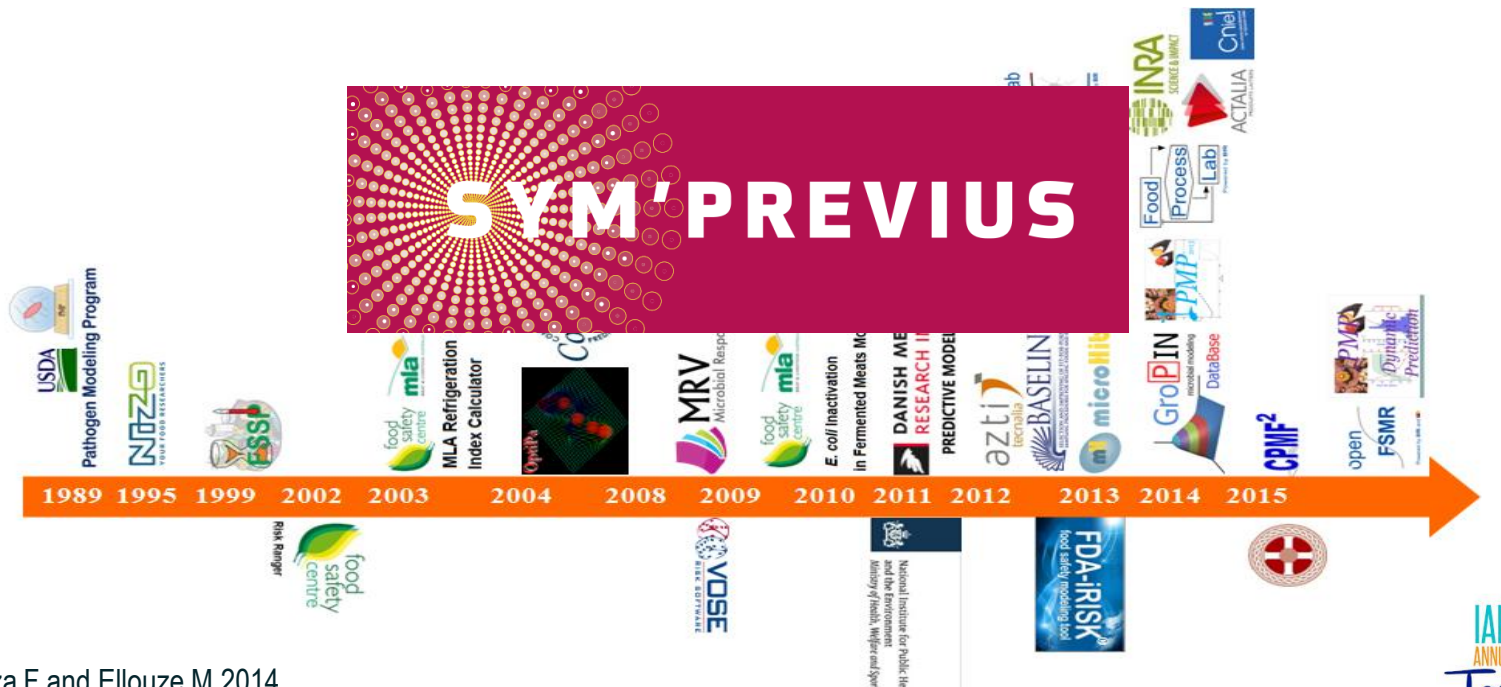


production/ process

Rationale

Predictive mathematical modeling describes & quantifies the evolution of microbial population taking into account intrinsic (pH, aw, organic acid …) and extrinsic characteristics (MAP, storage …)

Already available decision making tools



Tenenhaus-Aziza F and Ellouze M 2014.
Software for predictive microbiology and risk assessment: a description and comparison of tools. Food Microbiol 45:290-299

Sym'Previus decision making tool

A generic & modular approach for bacterial growth/ inactivation modeling in food

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A Modular Concept

$$\mu_{max} = \mu_{opt} \; \gamma(T)\; \gamma(a_w)\; \gamma(pH)\; \gamma(AH)\; \gamma(interaction)$$

Environmental factors

- cardinal values for each environmental factor (Rosso *et al.* 1995)
- γ functions for separate effect and interactions (Zwietering *et al.* 1996)

- ☑ deep strain characterization
- ☑ factors with biological significance
- ☑ μopt accounts for food matrix
- ☑ modular concept

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How to evaluate food shelf-life?

How to assess growth of a given micro-organism in a given food for given conditions of storage?

1. • Strain selection

Growth cardinal values

2. • impact of food matrix

1 challenge test

3. • Food shelf-life evaluation

Storage conditions

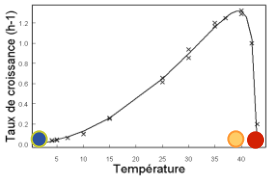
SYM'PREVIUS

How to evaluate food shelf-life?

Growth cardinal values account for the impact of environmental conditions on growth of a given strain …

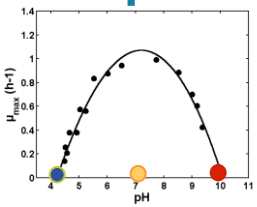
Growth cardinal values: minimal, optimal and maximal values of environmental factors impacting microbial growth (Rosso *et al.* 1995)

T°



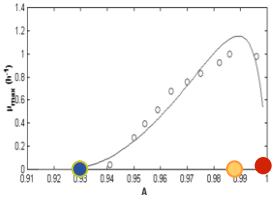
T_{min} T_{opt} T_{max}

pH



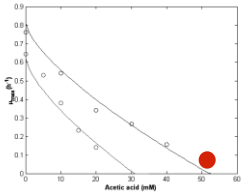
pH_{min} pH_{opt} pH_{max}

aw



aw_{min} aw_{opt} aw_{max}

acid



alpha MIC

SYM'PREVIUS

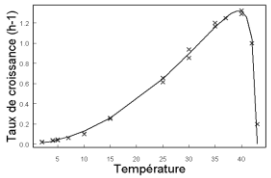
How to evaluate food shelf-life?

Growth cardinal values account for the impact of environmental conditions on growth of a given strain …

Growth cardinal values: minimal, optimal and maximal values of environmental factors impacting microbial growth (Rosso *et al.* 1995)

γ concept: impact of environmental factors on microbial growth (Zwietering *et al.* 1996)

Ex:T°



$\mu_{max} = \mu_{opt} \cdot \gamma_T$

$0 < \gamma_T < 1$

$$\gamma_T = \frac{(T - T_{min})^2 (T - T_{max})}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)\right]}$$

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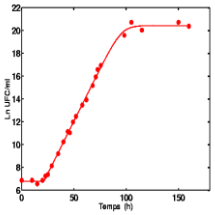
How to evaluate food shelf-life?

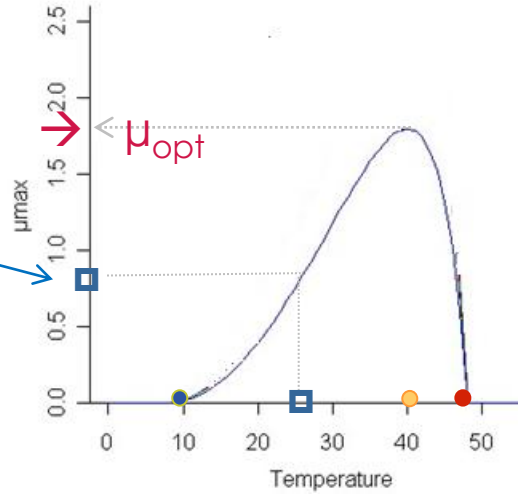
Growth kinetics determined after artificial inoculation of the strain of interest in food (challenge test) account for the impact of food on the contaminant growth

μmax: growth rate calculated during the challenge test



→ μ_{max}

Growth kinetic determined for given conditions (T°)



SYM'PREVIUS

How to evaluate food shelf-life?

2

Growth kinetics determined after artificial inoculation of the strain of interest in food (challenge test) account for the impact of food on the contaminant growth

μmax: growth rate calculated during the challenge test

μopt: growth rate estimated for optimal conditions. This is specific on a couple contaminant/food and is used for mathematical modeling

$$\mu_{max} = \mu_{opt}\ \gamma(T)\ \gamma(aw)\ \gamma(pH)\ \gamma(AH)\ \gamma(interaction)$$

Impact of food matrix

Impact of environmental factors on bacterial growth

SYM'PREVIUS

How to evaluate food shelf-life?

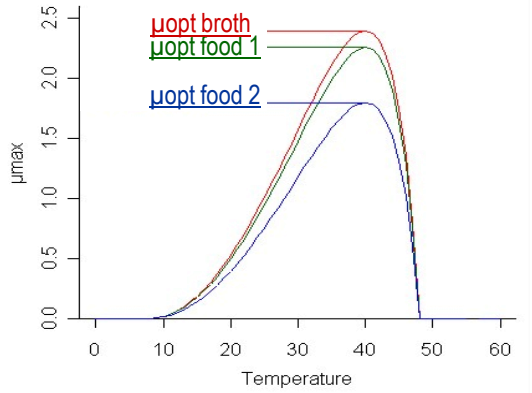
2

Growth kinetics determined after artificial inoculation of the strain of interest in food (challenge test) account for the impact of food on the contaminant growth

μmax: growth rate calculated during the challenge test

μopt: growth rate estimated for optimal conditions. This is specific on a couple contaminant/food and is used for mathematical modeling

2 food with similar characteristics (T°, pH, a_w, inhibitor), may not support growth in a similar way.



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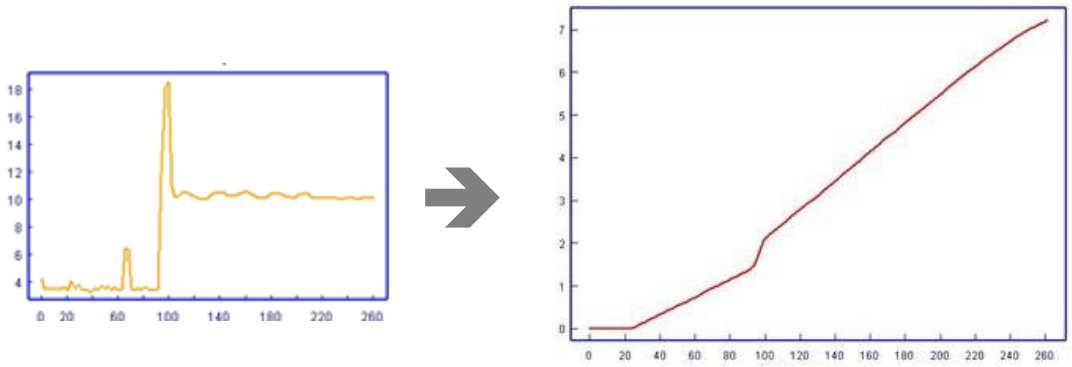
How to evaluate food shelf-life?

For this given strain/food combination, growth simulation along shelf-life are possible for various scenarii of interest in a few clics

Scenario of industrial relevance: static or dynamic conditions of storage to quantify the impact of a change in formulation, batch variability, storage of the products.



A Modular Concept

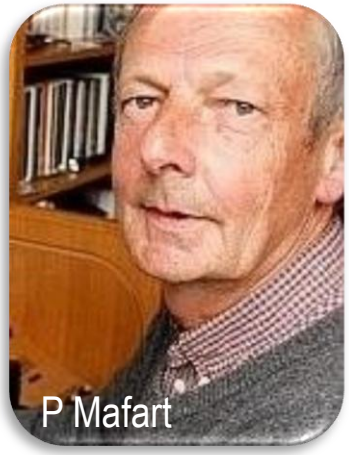
$$\mu_{max} = \mu_{opt} \; \gamma(T)\; \gamma(a_w)\; \gamma(pH)\; \gamma(AH)\; \gamma(interaction)$$

Environmental factors

- cardinal values for each environmental factor (Rosso *et al.* 1995)
- γ functions for separate effect and interactions (Zwietering *et al.* 1996)

- ☑ deep strain characterization
- ☑ factors with biological significance
- ☑ μopt accounts for food matrix
- ☑ modular concept

A Modular Concept



$$\text{apparent heat resistance} = 1/\delta^*_{max}\ \lambda(T)\ \lambda(pH)\ \lambda(a_w)\ \lambda(T')\ \lambda(pH')\ \lambda(a_w')$$

Environmental factors

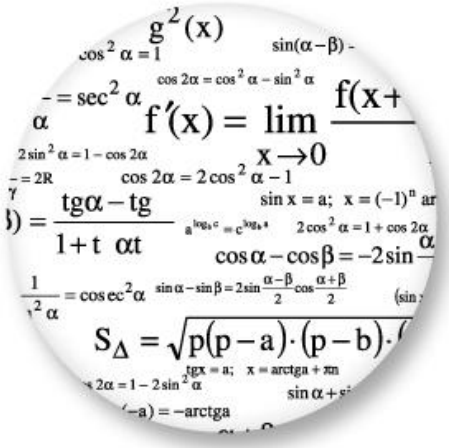
treatment & recovery'

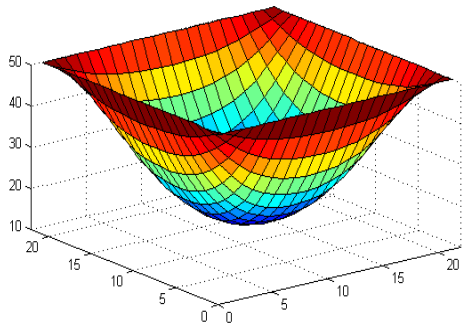
- bacterial population reduction (δ) after heat exposure (Mafart *et al.* 2000)
- λ functions for separate effect inspired from γconcept (Mafart *et al.* 2002)

- ☑ deep strain characterization
- ☑ factors with biological significance
- ☑ modular concept

from research to an operational tool

Sym'Previus is a decision making tools are only friendly user interfaces using published and scientifically recognized mathematical models to support FBO, technical institution, food safety authorities and other stakeholders.





workflow

Sym'Previus organisation & collaborative inputs

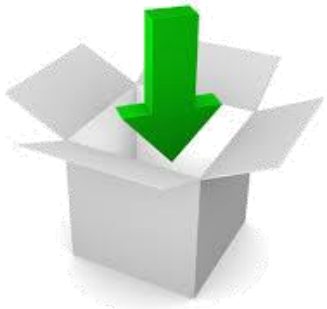
- GIS for industrial and policy orientation
- CST for scientific and technical issues & GAT working groups
- Operational cell for computer encoding and developments incrementation

Mathematical models,
Biological parameters …

GAT

CST

CST



Data available for members

workflow

Sym'Previus organisation & collaborative inputs

- GIS for industrial and policy orientation
- CST for scientific and technical issues & GAT working groups
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Operational cell since 2000

just try it !

130€/year: individual membership needed to access Sym'Previus and ensure quality, system maintenance and support this collaborative network

Within the frame of FOODSTA webinar series, just try Sym'Previus with a one month free access

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valid from 14june-14july 2017



Training
Audit - Consulting

Research & Innovation

Sym'Previus decision making tool

The toolbox

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SYM'PREVIUS

the toolbox

A decision making tool for food quality& safety gathering several modules for several applications, to support FBO, technical institution, food safety authorities and other stakeholders.

- microbial food hazards identification & CCP determination
- food shelf-life validation for adjusted food quality & safety
- thermal process optimisation
- better outreach & quantification of microbial behavior

… ok, but now?

SYM'PREVIUS

the toolbox

A minimum of input data for a maximum of results!

Industrial inputs:

Food properties
(pH, a_w …)

Food process
(T, t, storage…)

In silico predictions on bacterial behavior in food

SYM'PREVIUS

the toolbox

A minimum of input data for a maximum of results!

- Test in a few clics various relevant scenarios using Sym'Previus database (microbiological data and mathematical equations)

- Sym'Previus database gathers both pathogenic & spoilage bacterial species for growth simulation and inactivation

L. monocytogenes, B. cereus, Salmonella, E. coli, S. aureus, C. botulinum, C. perfringens,

B. amyloliquefaciens, B. licheniformis, C. freundii, E. faecium, L. casei, L. sakei, Le. Mesenteroides, P. fluorescens, P. putida, S. marescens…

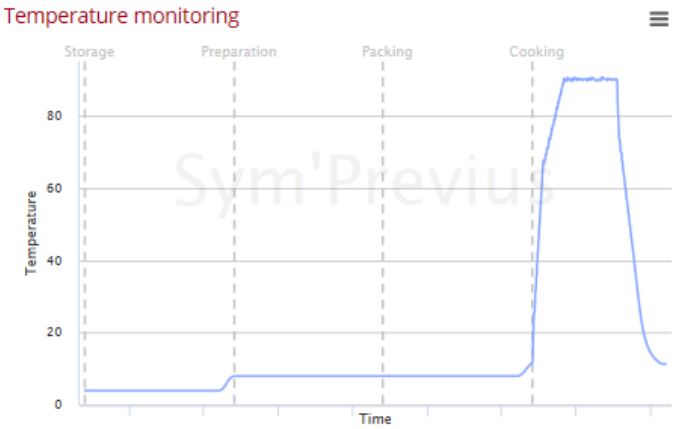
Or any customised strain of your interest !

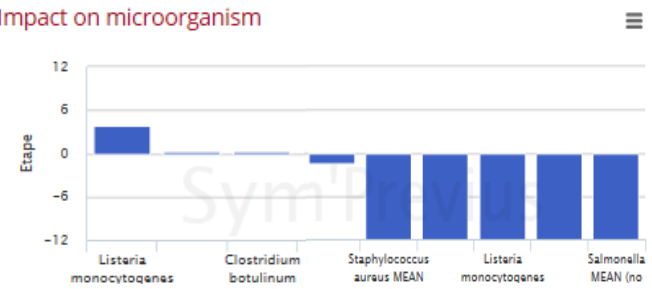
HACCP

Objectives: identify CCP along food production process for given spoilage or pathogenic bacteria.

Input data: temperature, a_w, pH process recordings

Output data: bacterial growth or destruction for each step of the process, considered independently



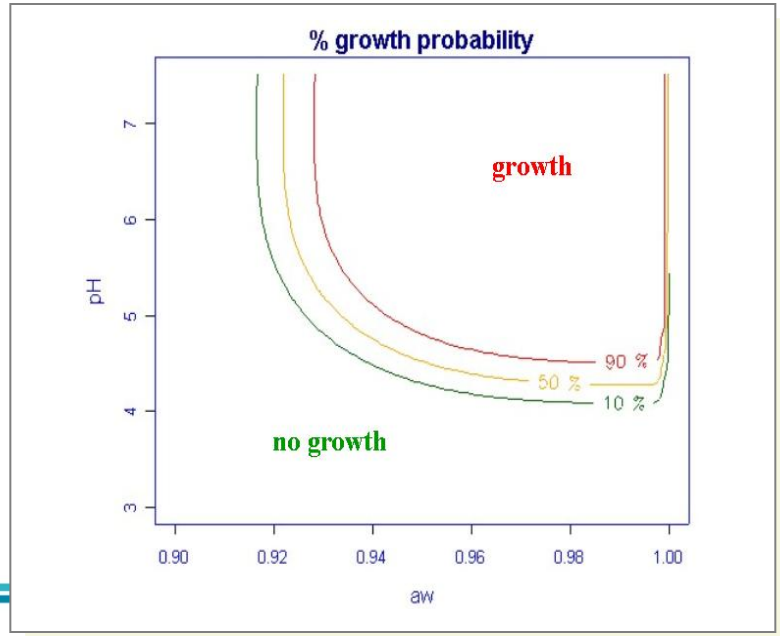


- growth/no growth boundaries

Objectives: determine boundary where growth of a given microorganism is possible based on food physico-chemical features

Input data:
Bacterial cardinal values *i.e.* minimal, optimal and maximal values of pH, a_w, T° for growth

Output data: boundary for bacterial growth/no growth

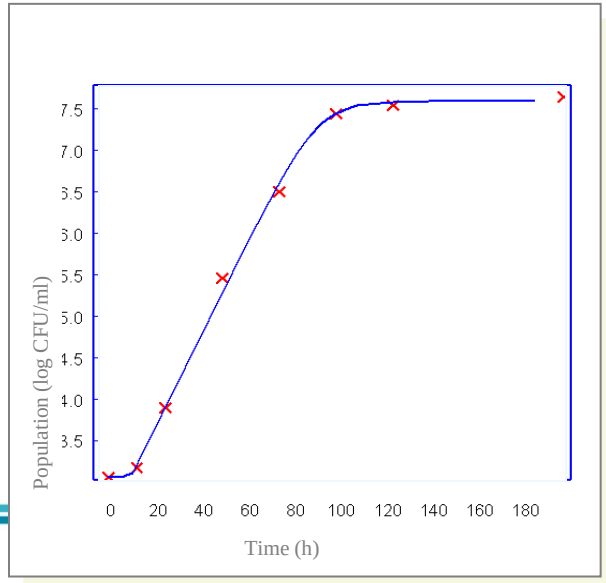


- growth fitting

Objectives: fit enumeration data & determine primary model parameters

Input data: Bacterial kinetics

Output data: primary model parameters, *i.e.* μmax, lag, No, Nmax

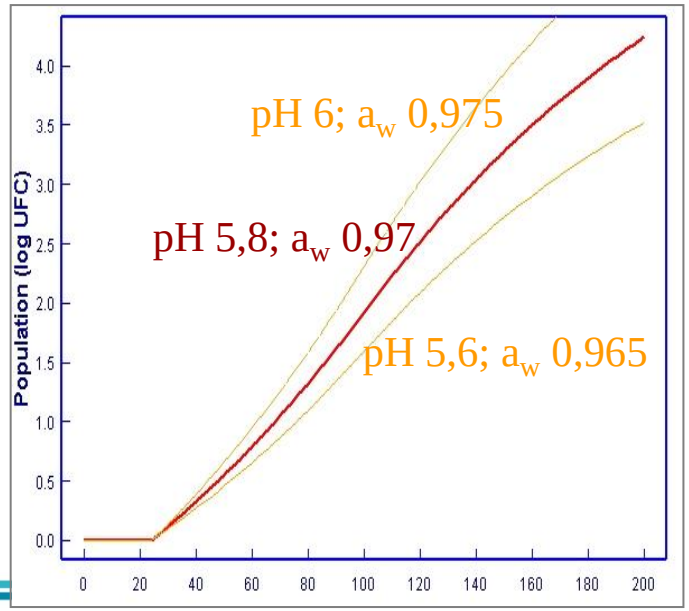


- growth simulation

Objectives: simulate *in silico*, growth for further conditions (formulation, process, storage …)

Input data: dynamic/static recordings of temperature; pH, a_w …

Output data: growth simulation

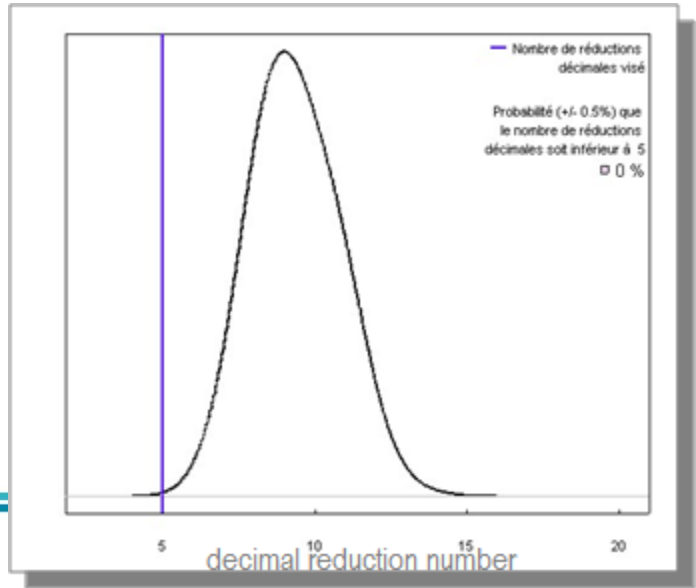


- heat destruction simulation

Objectives: validate heat treatment taking into account heat penetration in food (f values), process parameters and targeted population reductions

Input data: bacterial kinetics, strain heat resistance and temperature profiles

Output data: decimal reduction (D or δ), surve shape (p), initial and final population



Sym'Previus decision making tool

Practical cases

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Production process of a rice pudding will be studied to

- identify hazards
- quantify process efficiency
- validate food shelf-life

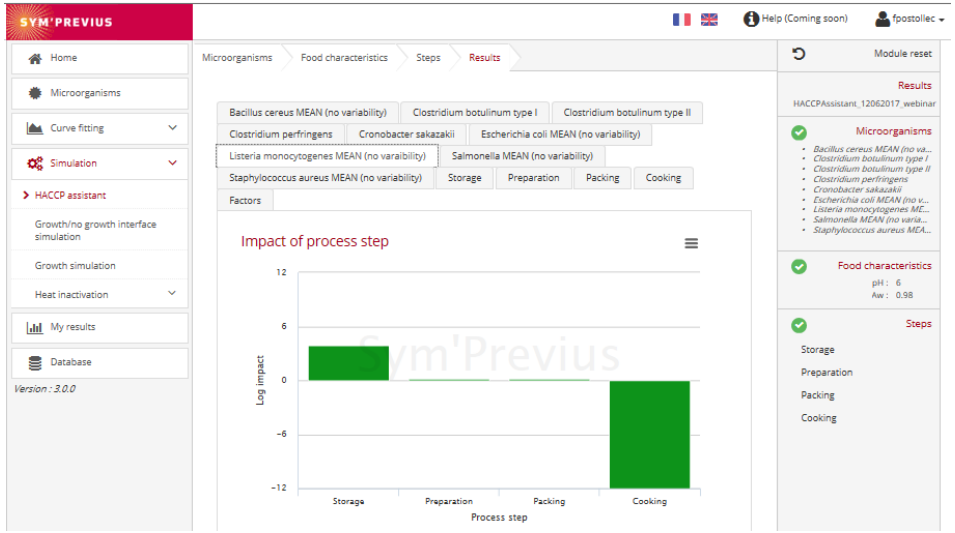
1. Identify bacterial hazards

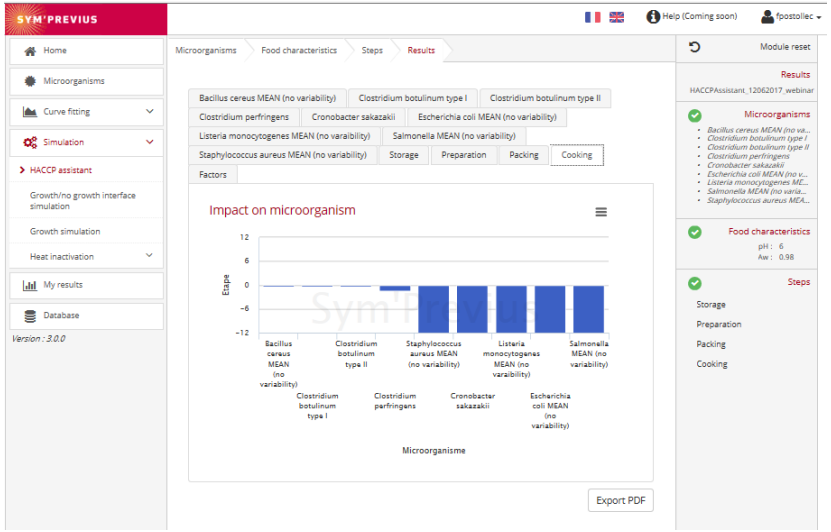
Raw material	2°C / 4 days
↓	
Mixing	8°C / 1 hour
↓	
Packing	
↓	
Heat treatment	90°C / 45 min
↓	
Fast cooling	up to 2°C
↓	
Storage / Distribution	4°C / 20 days
End product	pH =6, a_w=0.98

SYM'PREVIUS

case study

1. identify bacterial hazards & CCP





- Cooking step is a CCP to control bacterial contaminants (vegetative cells)
- Destruction of sporeformers will not be ensured

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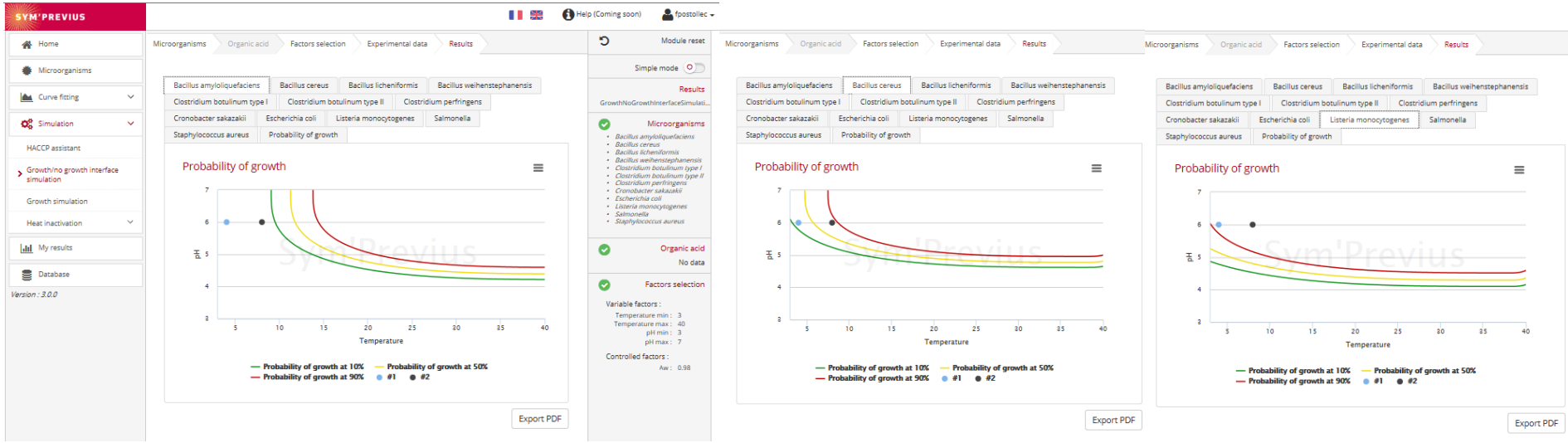
case study

1. identify bacterial hazards & CCP

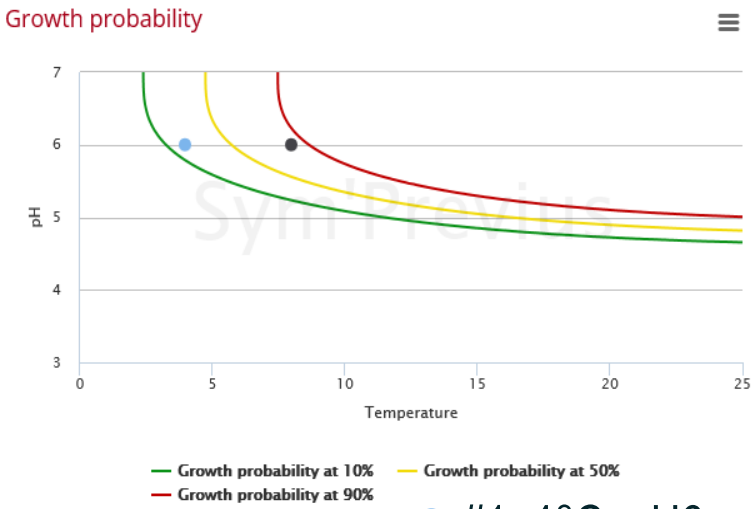


- Ensure no contamination is possible after CCP
- Food formulation is supporting growth of some sporeformers, in particular *B. cereus*

SYM'PREVIUS

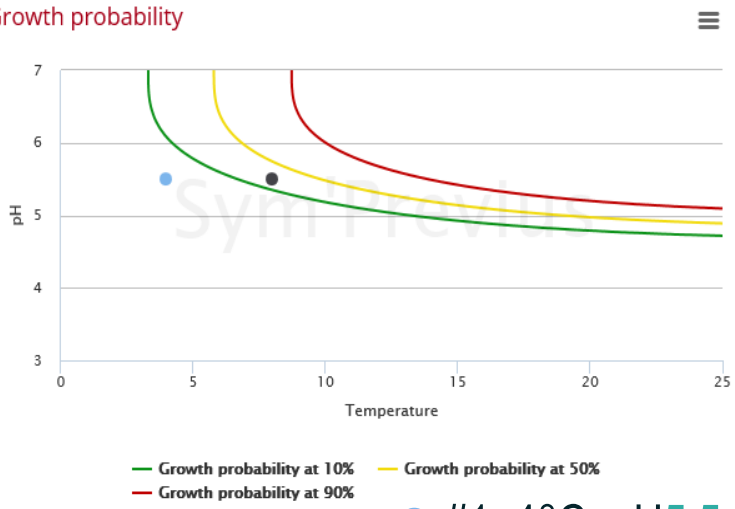
case study

What impact of a formulation change ?



9 strains, a_w = 0.98

- #1: 4°C, pH6
- #2: 8°C, pH6



9 strains, a_w = 0.97

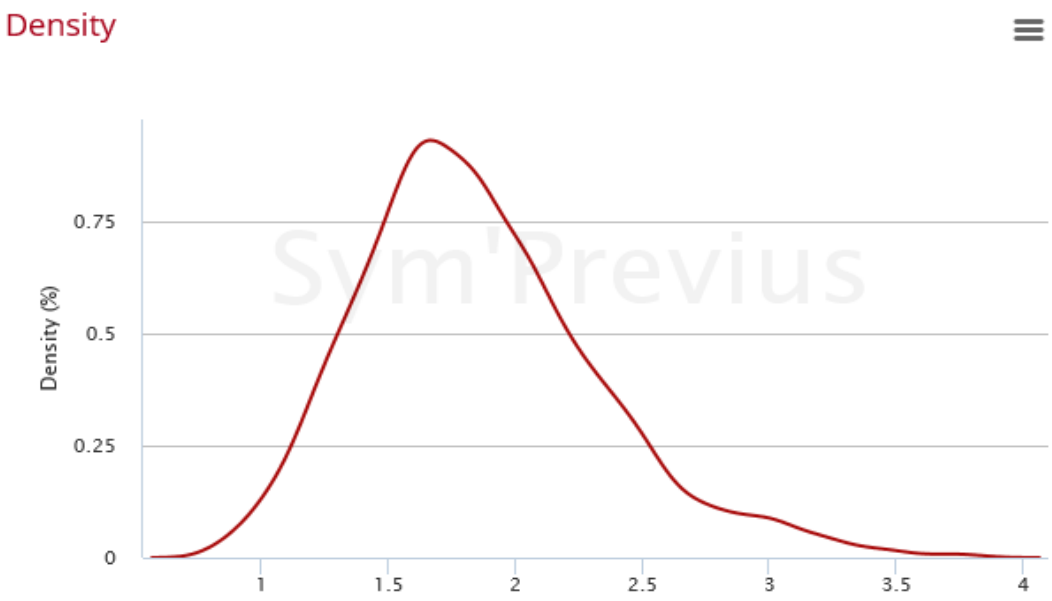
- #1: 4°C, pH5.5
- #2: 8°C, pH5.5

Unless strictly stored at 4°C, food formulation supports growth of *B. cereus*

2. quantify process efficiency

Raw material	2°C / 4 days
↓	
Mixing	8°C / 1 hour
↓	
Packing	
↓	
Heat treatment	90°C / 45 min
↓	
Fast cooling	up to 2°C
↓	
Storage / Distribution	4°C / 20 days
End product	pH =6, a_w=0.98

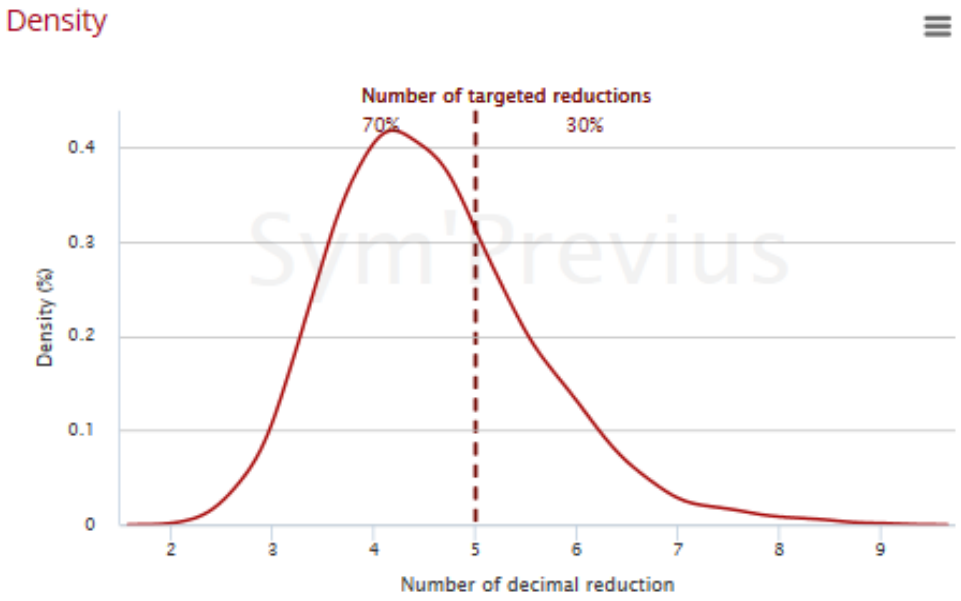
2. Simulation of population destruction



90°C-45min
Targeted reduction:5
B. cereus strain A

➲ Destruction of *B. cereus* strain A is ensured by this given heat treatment, but wide diversity is observed

2. Simulation of population destruction



90°C-45min

Targeted reduction:5

B. cereus strain A

→ decimal reduction ~2

B. cereus strain C

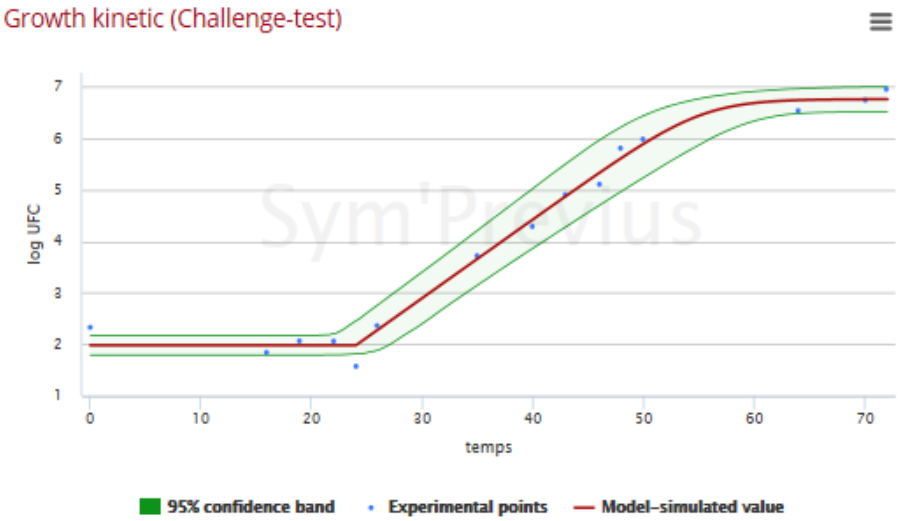
→ decimal reduction <5

➲ Depending of contaminating strain, targeted population reduction is not achieved !

3. validate food shelf-life

Raw material	2°C / 4 days
↓	
Mixing	8°C / 1 hour
↓	
Packing	
↓	
Heat treatment	90°C / 45 min
↓	
Fast cooling	up to 2°C
↓	
Storage / Distribution	4°C / 20 days
End product	pH =6, a_w=0.98

3. growth simulation during shelf-life

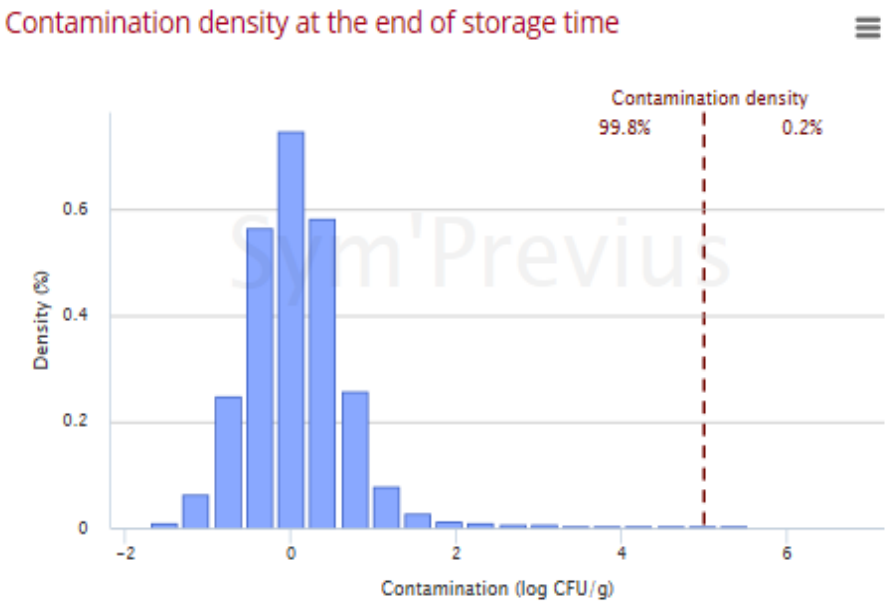


Estimated primary parameters

	Estimated values	Standard Deviation
No (Log UFC/g)	1.98	0.1
Nmax (Log UFC/g)	6.75	0.13
μmax (/h)	0.352	0.025
lag (h)	24	1.47
tg (h)	1.981	0.143

Based on 1 challenge test, growth rate is determined for a given combination contaminant/foodstuff

3. growth simulation during shelf-life

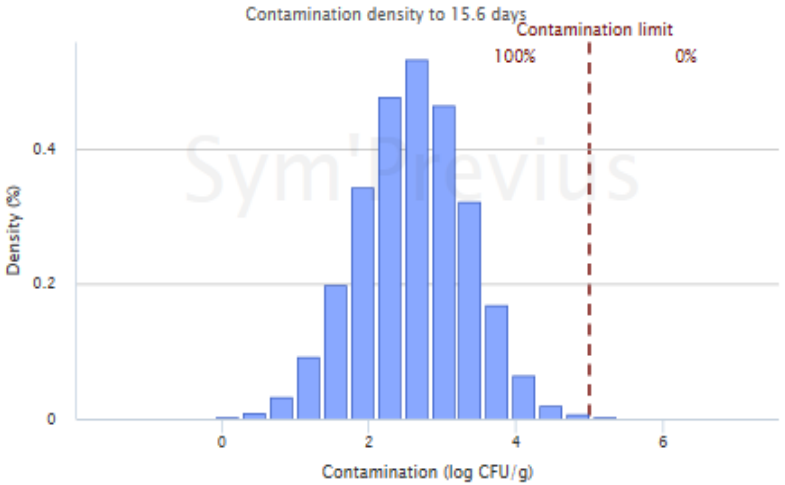


temperature storage scenario:
1/3 @4°C, 2/3 @8°C
during 20 days

➲ If refrigerated storage condition is ensured, food shelf-life of 20days is validated

3. growth simulation during shelf-life

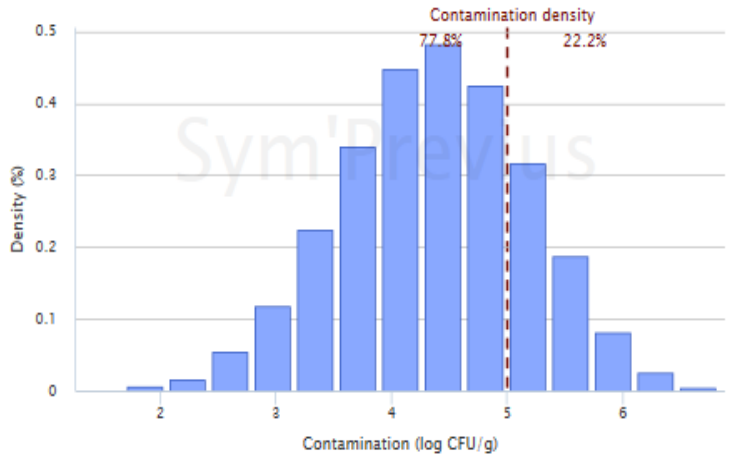
Contamination density



For less stringent refrigeration conditions or the contamination of psychrotolerant strain, *B. cereus* population will develop during shelf-life

3. growth simulation during shelf-life

Contamination density at the end of storage time



For less stringent refrigeration conditions or the contamination of psychrotolerant strain, *B. cereus* population will develop during shelf-life

➲ In a few clic, evolution of targeted population may be predicted for relevant industrial scenarii

take home message

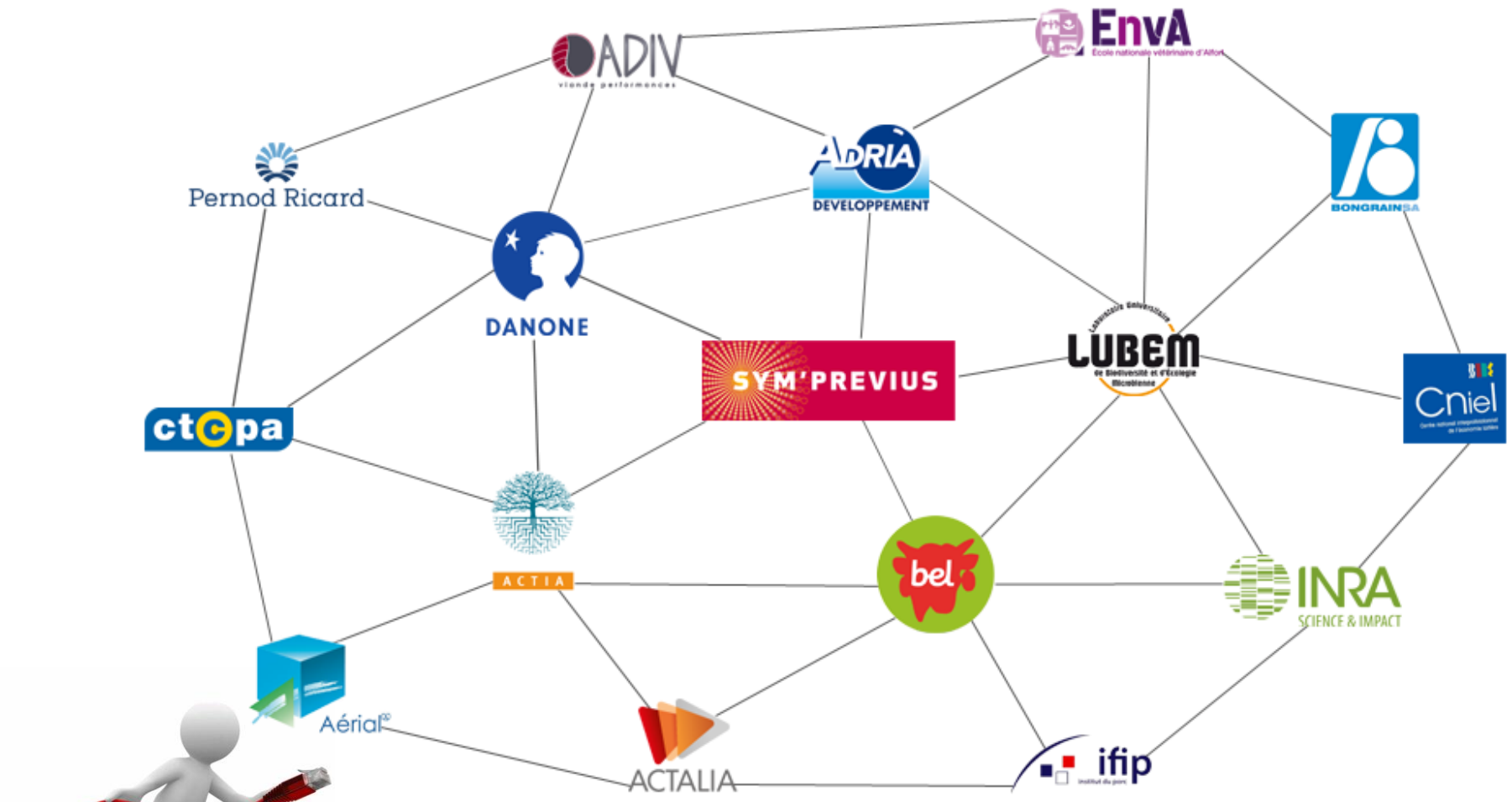
01 Mathematical predictive models are available & recognized by scientific community

02 Sym'Previus complies with regulation and offers a quality of input and output data via a web-based plateform and network of expertise

03 Available & generic approach to assess bacterial growth, survival, inactivation in food to ensure food safety & quality

want to support & join the network?

Sym'Previus® operational unit | symprevius@adria.tm.fr | +33 2 98 10 18 76



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