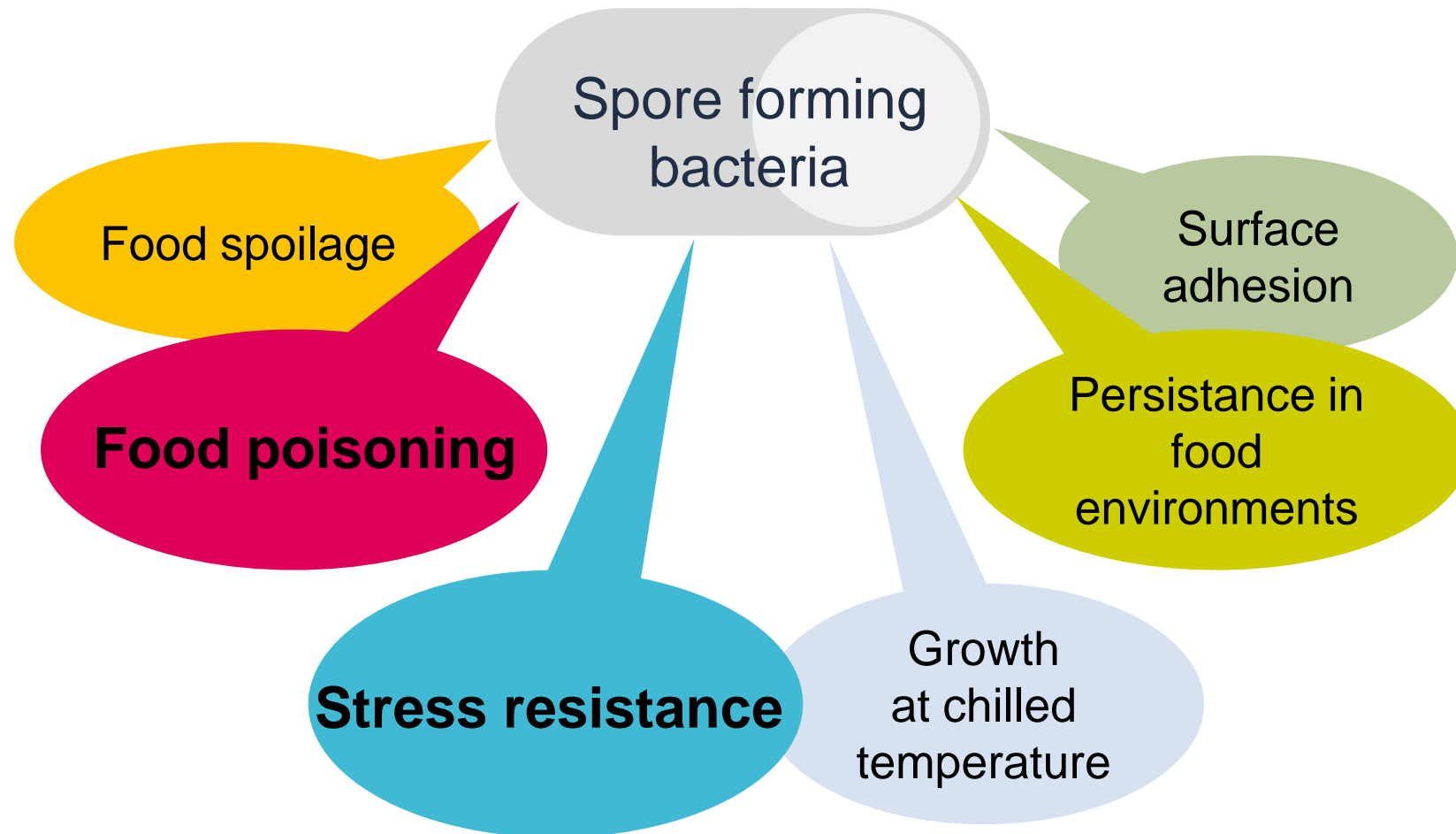


Determination of acid-stress bacterial resistance biomarkers of *Bacillus weihenstephanensis*

ICPMF7, Dublin 2011

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-Foodborne contaminants and industrial issues-



✓ Hazard for heat-processed RTE stored at refrigerated temperature

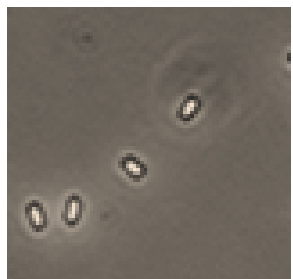
-Foodborne contaminants and industrial issues-



Acid stress

Sporulation

Low spore formation
under acid conditions



➤ E. Baril work
Poster # 28 and 30

Vegetative growth/ survival

Bacterial resistance



Aim: Identification of molecular biomarkers that could predict the acid stress resistance of *B. weihenstephanensis* KBAB4

Biomarkers: Characteristic that is **objectively measured** (with satisfactory accuracy and reproducibility) and evaluated **as an indicator** biologic processes (Atkinson *et al.*, 2001).

Predictive microbiology

✓ Quantification of
acid bacterial resistance
Biological process

Molecular biology (RT-qPCR)

✓ Quantification of
genes expression
Reproducible biomarker

Integrative approach

✓ Correlation genes expression
and bacterial resistance

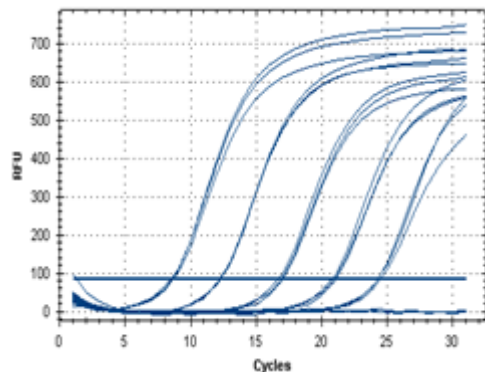
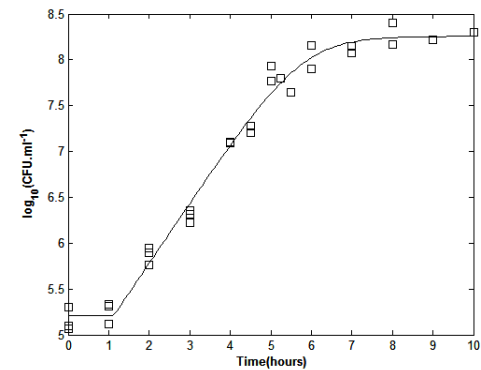
-Control and optimization-

- Inoculum :
3 successive cultures to minimize physiological states variation

- Bacterial counts:
Satisfactory repeatability (standard deviation < 0.5 log)

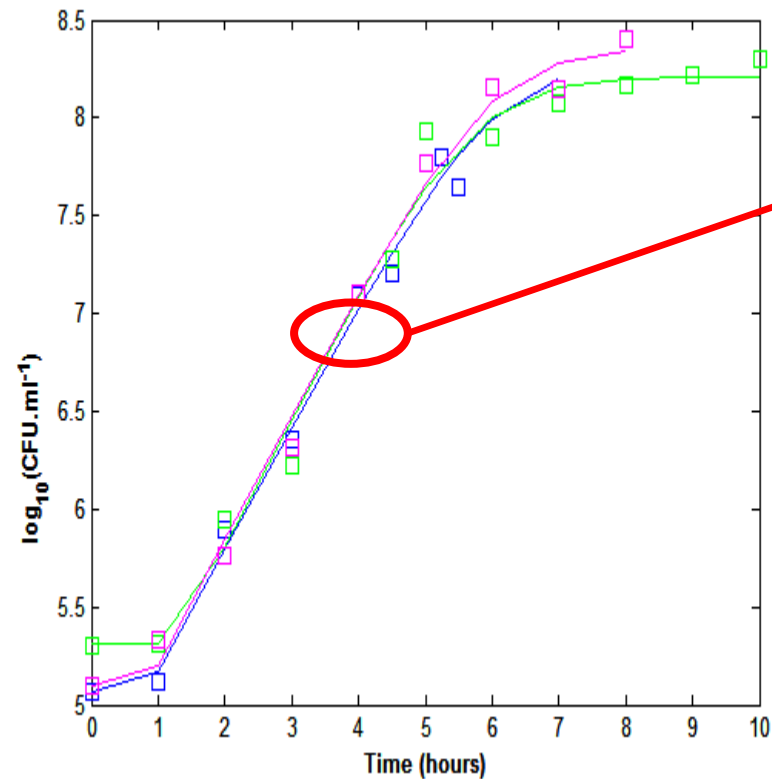
- RNA extraction :
Satisfactory reproducibility and extract quality (RQI>5)

- RT-qPCR:
Cq variation for similar sample < 1Cq
Satisfactory experimental variation

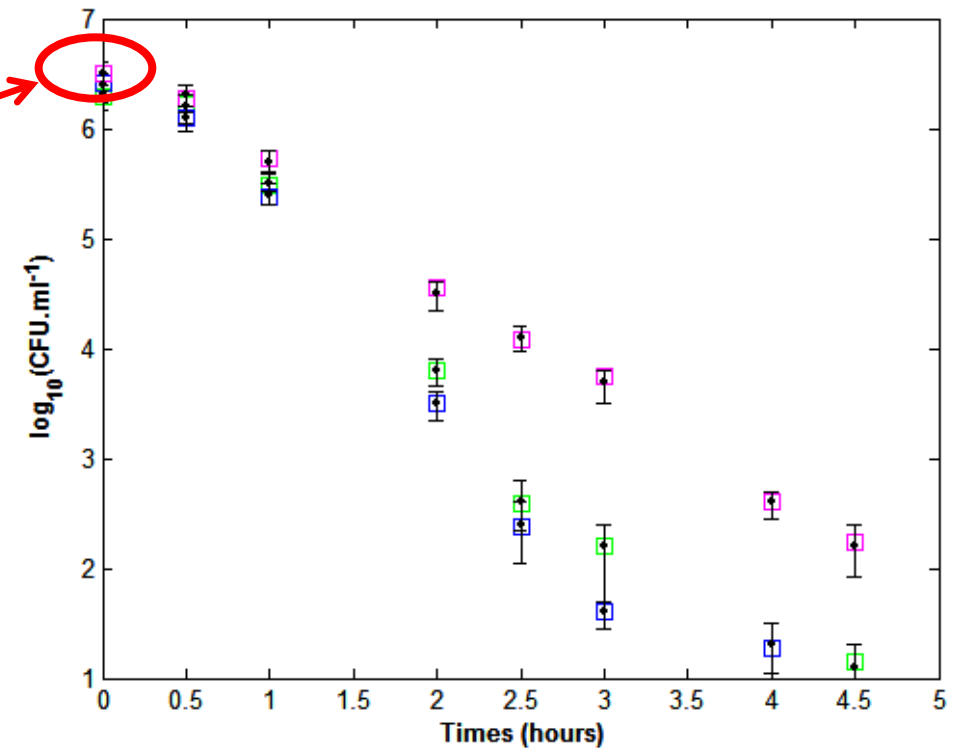


- Acid inactivation -

BHI broth pH 7.2, 30°C, 100 rpm



BHI broth pH 4.6, 30°C, 100 rpm

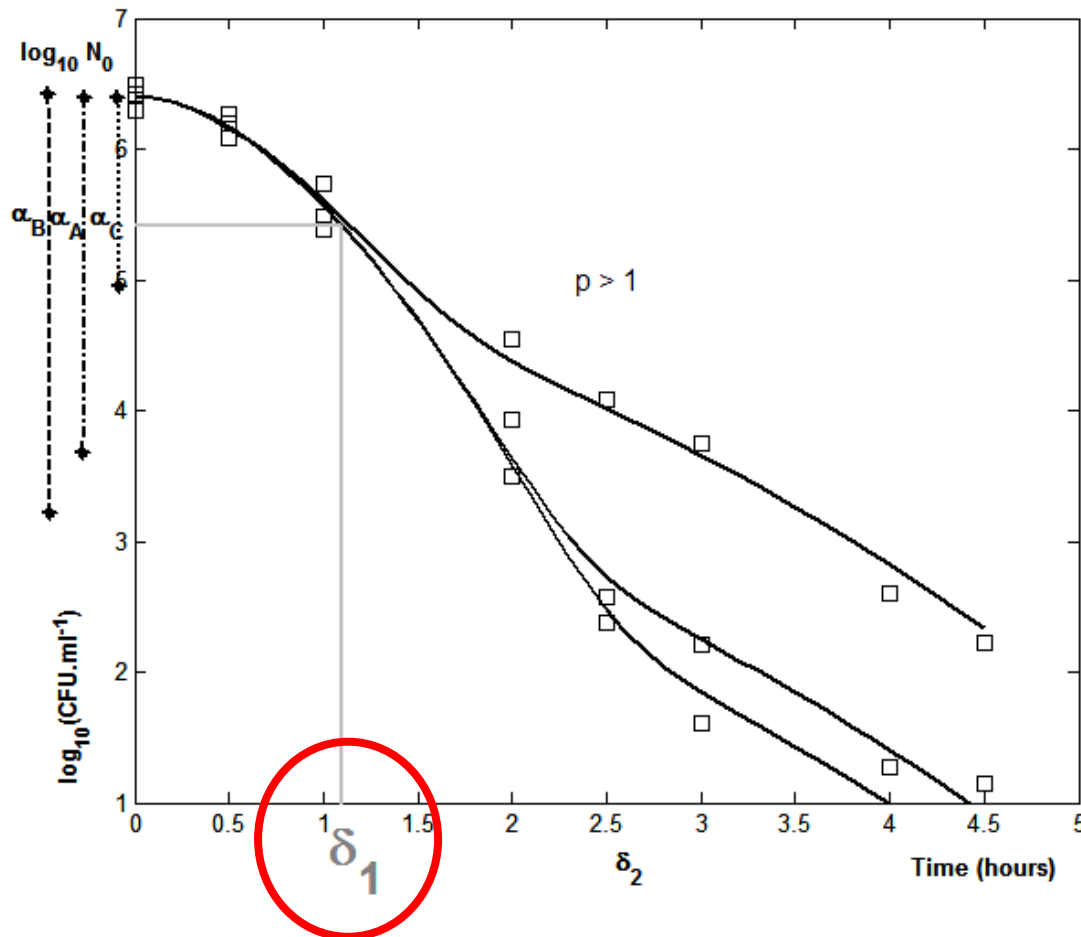


Mid exponential cells (optimal conditions) submitted to acid inactivation (pH 4.6)
Sampling performed for each inactivation time : survival counts and RT-qPCR

-Mixed Weibull model-

1. Acid bacterial resistance quantification

2 subpopulations different acid resistances



■ Subpopulation 1
 $\delta_1 = 1.10 \pm 0.20$ (h)

■ Subpopulation 2
 $\delta_2 = 2.61 \pm 0.56$ (h)

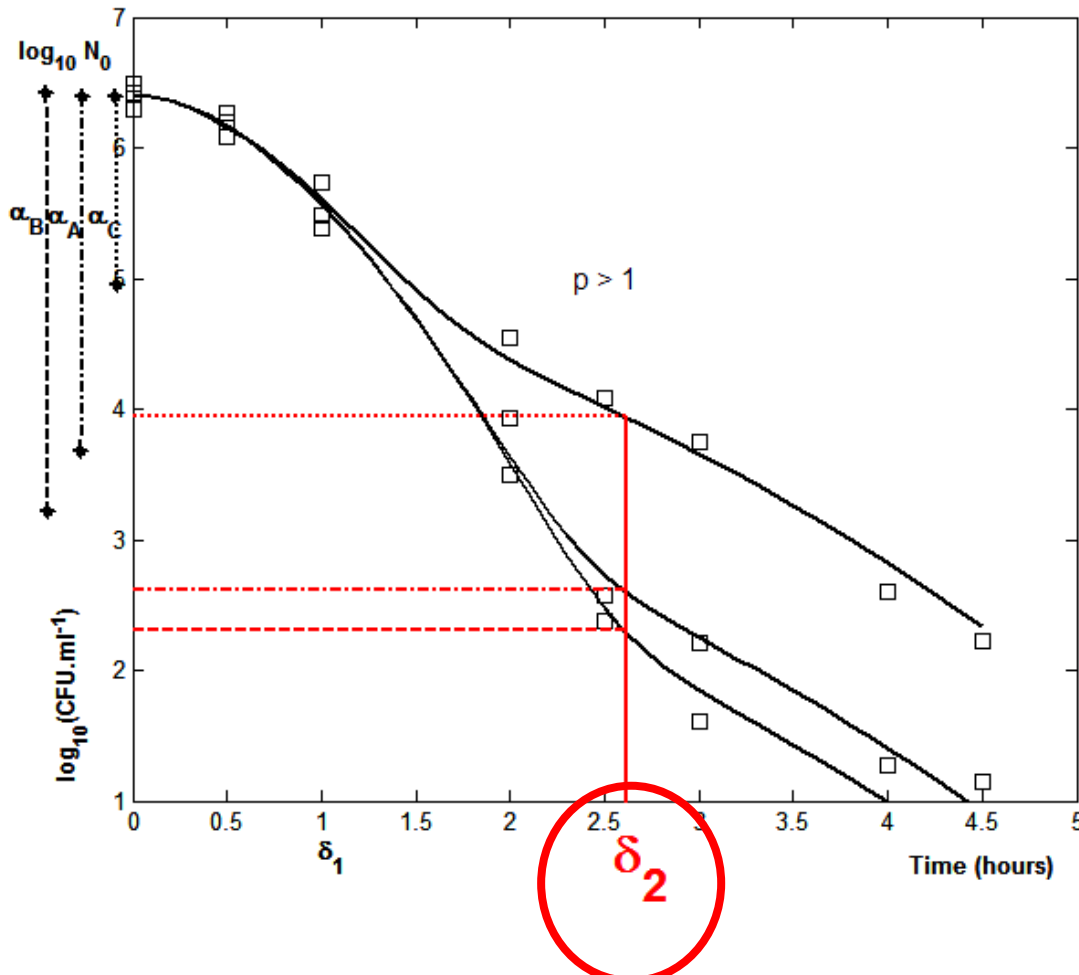
Other estimated parameters

- $\log_{10} N_0 = 6.41 \pm 0.20$ (CFU.ml⁻¹)
- $P = 1.75 \pm 0.42$
- $\alpha_A = 2.89 \pm 0.46$
- $\alpha_B = 3.31 \pm 0.46$
- $\alpha_C = 1.46 \pm 0.43$

-Mixed Weibull model-

1. Acid bacterial resistance quantification

2 subpopulations different acid resistances



■ Subpopulation 1 : most sensitive subpopulation
 $\delta_1 = 1.10 \pm 0.20$ (h)

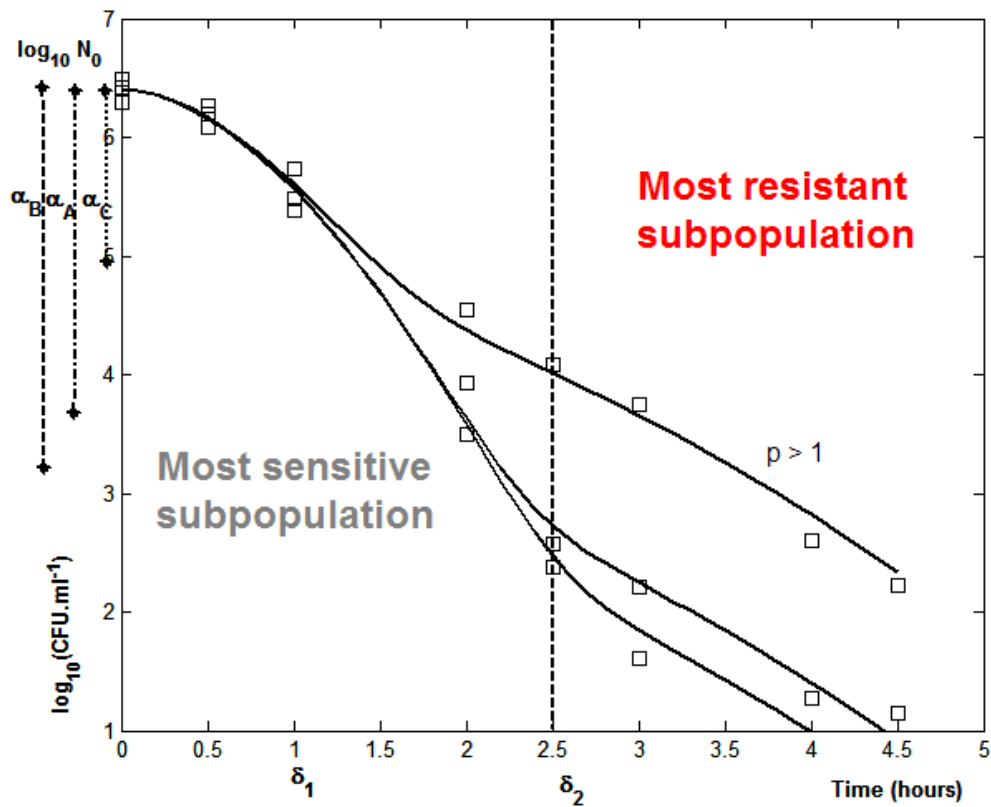
■ Subpopulation 2 : most resistant subpopulation
 $\delta_2 = 2.61 \pm 0.56$ (h)

Other estimated parameters

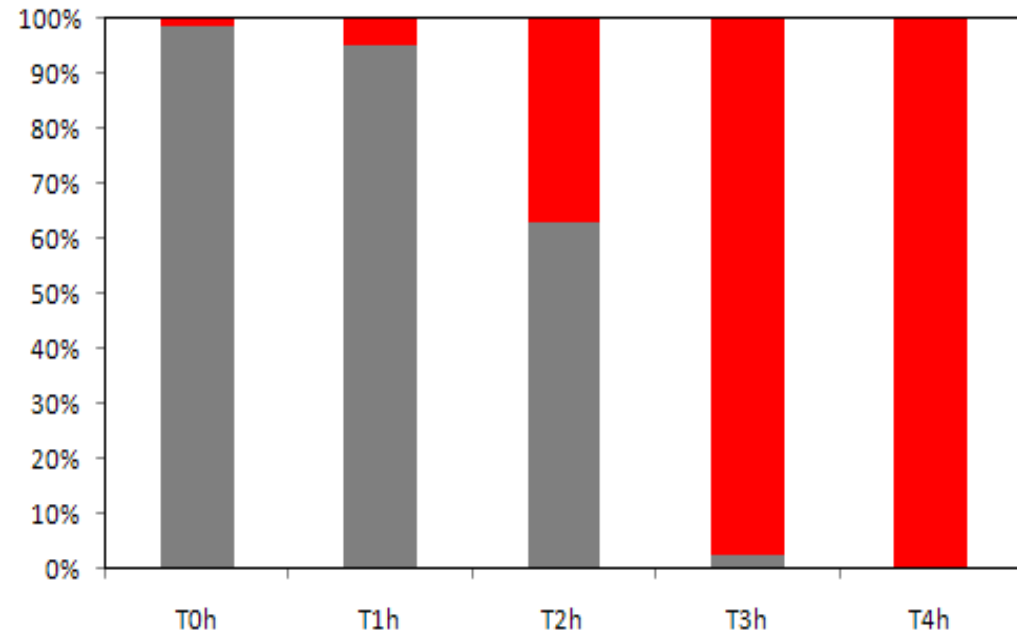
- $\log_{10} N_0 = 6.41 \pm 0.20$ (CFU.ml⁻¹)
- $P = 1.75 \pm 0.42$
- $\alpha_A = 2.89 \pm 0.46$
- $\alpha_B = 3.31 \pm 0.46$
- $\alpha_C = 1.46 \pm 0.43$

-Mixed Weibull model-

2. Proportion of the two subpopulations

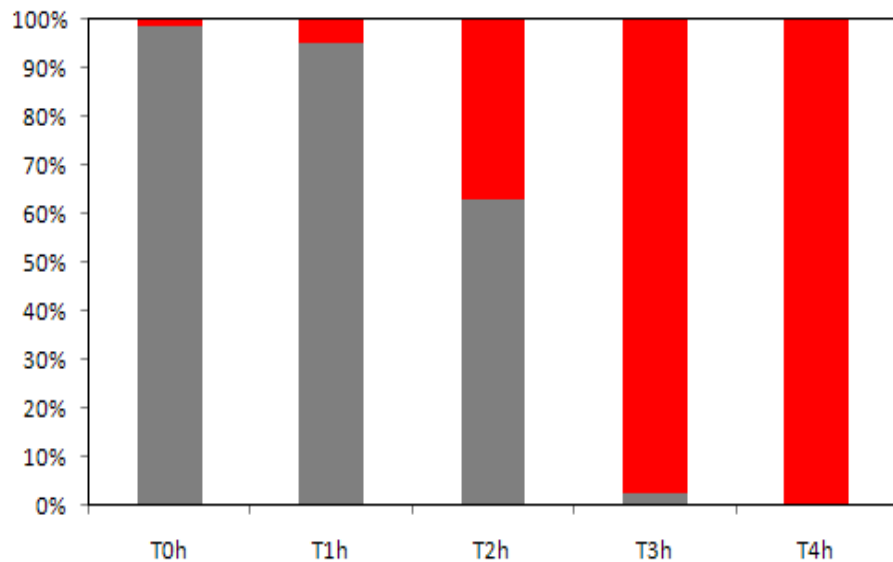


...within the triplicate

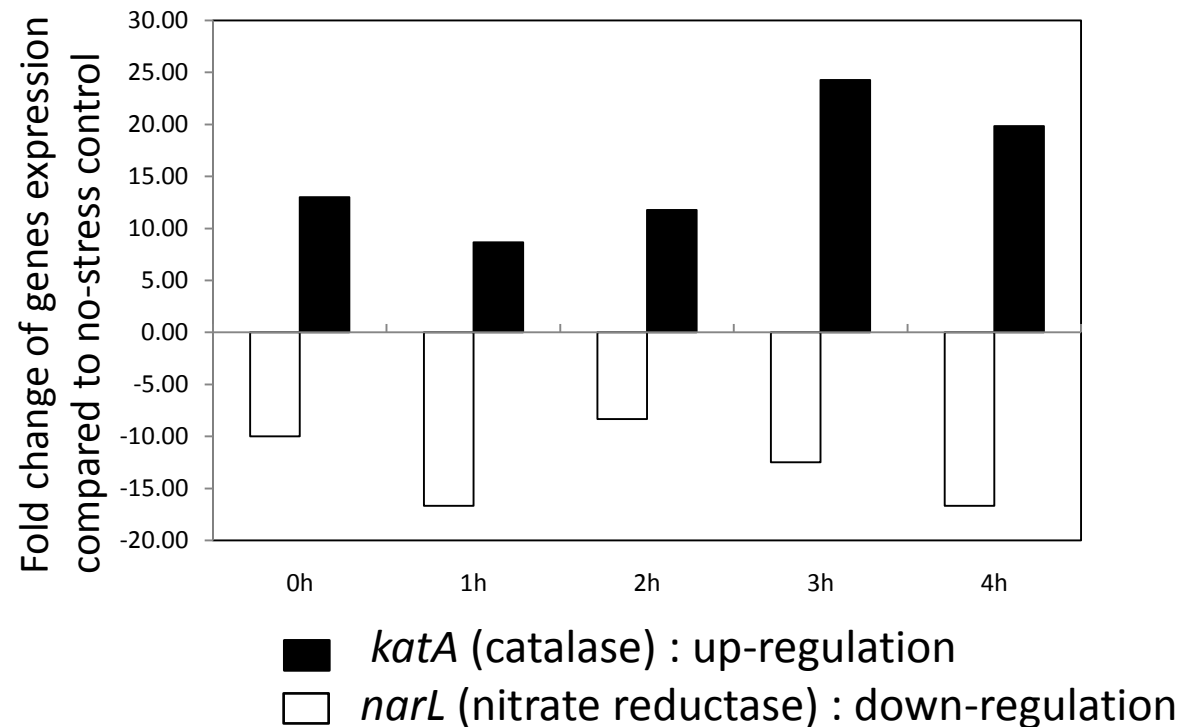


-Integrative approach-

Subpopulations with 2 acid-resistance
within the triplicate



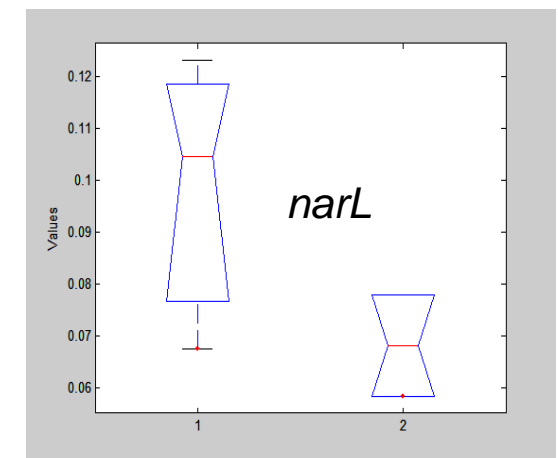
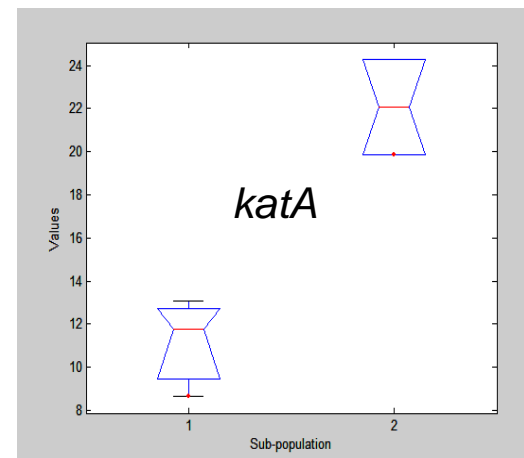
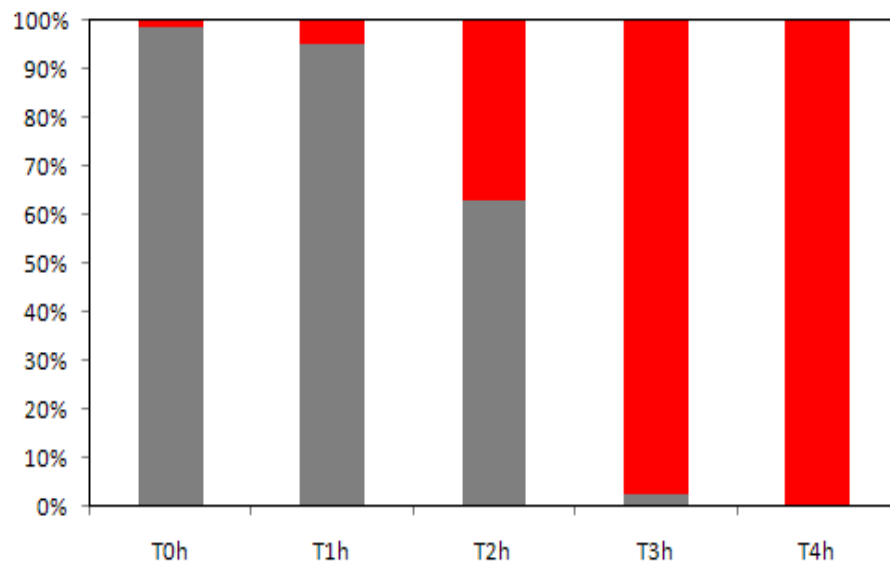
RT-qPCR biomarkers quantification
within the triplicate



✓ *katA* is a potential biomarker of acid bacterial resistance, in studied conditions 10

-Integrative approach-

Is there is any difference between the gene expression of the two subpopulations?



ANOVA one way :

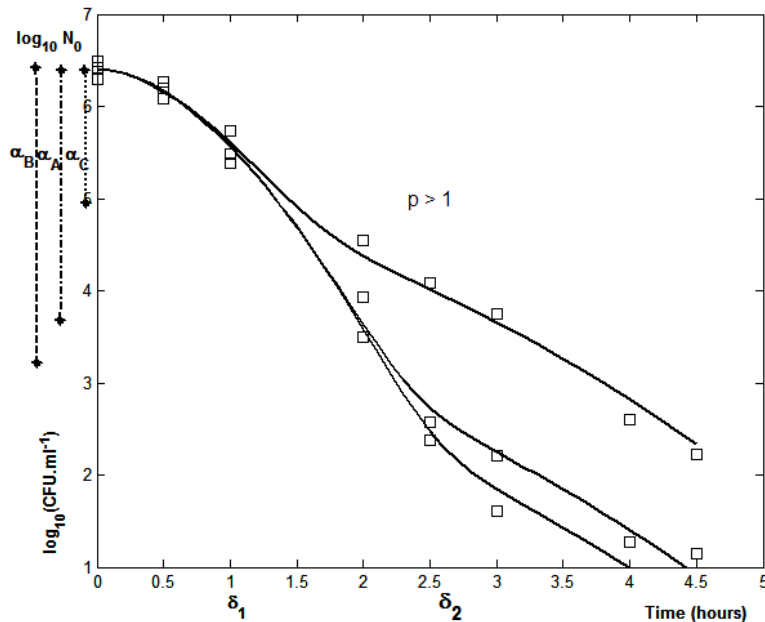
katA significant variation ($p < 0.05$)

narL no significant variation ($p > 0.05$)

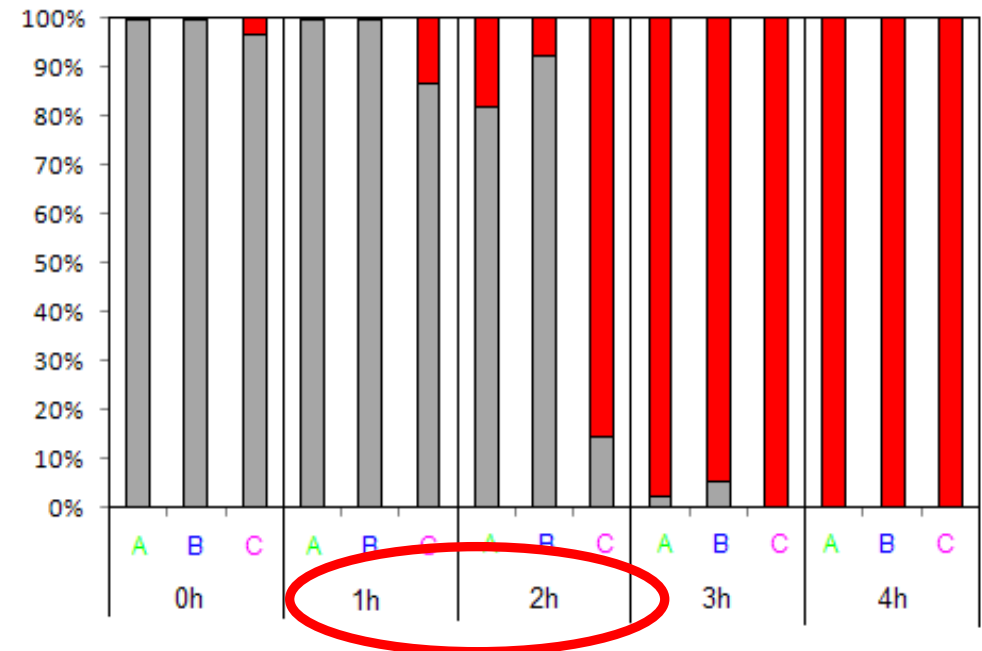
✓ Higher expression of *katA* in the most resistant subpopulation

-Integrative approach-

One replicate is more resistant than the other
Replicate C



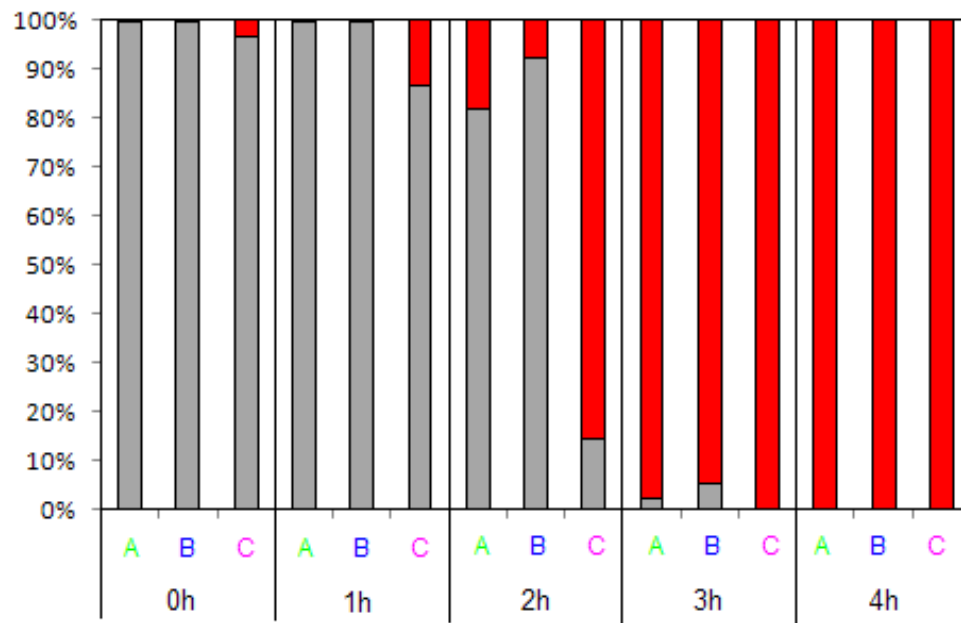
Proportion of the two subpopulations within
each replicate of the triplicate



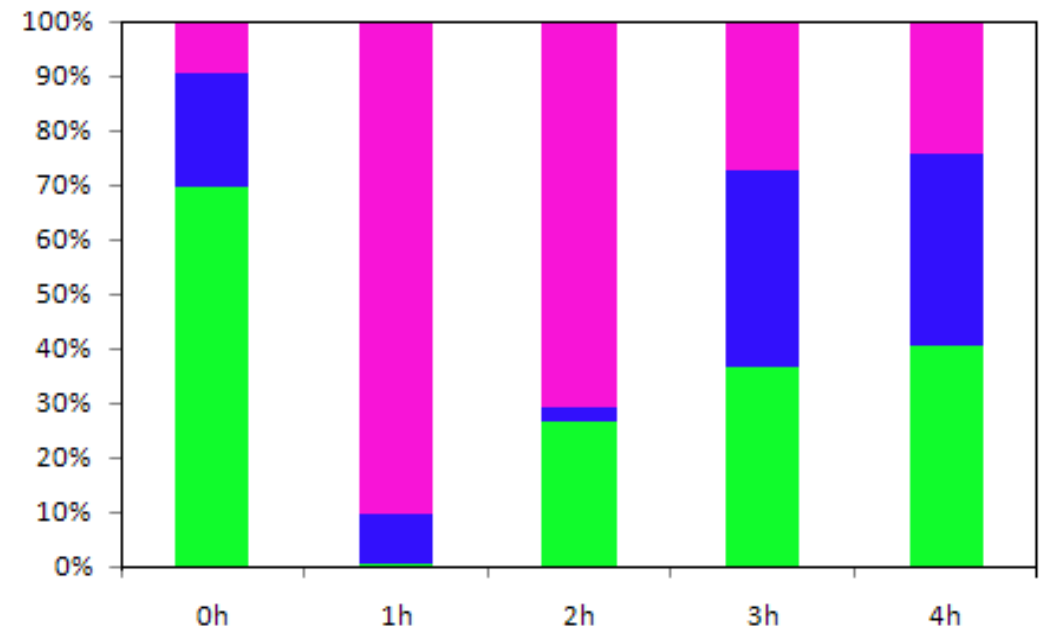
✓ At 1 and 2h, the proportion of the most resistant population is more important in the replicate C than in the other replicates

-Integrative approach-

Proportion of the two subpopulations within each replicate of the triplicate



Proportion of the *katA* gene expression quantification given by each replicate



✓ At 1 and 2h, the proportion of *katA* gene expression is mainly given by the replicate C (~90 and 70%)

Determination of acid-stress bacterial resistance biomarkers of *Bacillus weihenstephanensis*

- ✓ **Correlation between gene quantification and acid resistance of 2 subpopulations**
- ✓ **Identification of potential acid resistance biomarker (*katA*)**
- ✓ **Possibility to use quantitative molecular biomarkers in bacterial behavior modelling**

Even though further investigations are needed,
this promising results highlight the possibility
to integrate bacterial physiological state using Omic biomarker
into microbial risk assessment

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I. Leguerinel, L. Coroller



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NUTRITION

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7th International Conference 2011
Predictive Modelling of Food Quality and Safety

$\mu_{max} = a(T - T_{min})$

$D = 0,21 \text{ min @ } 121,1 \text{ }^\circ\text{C}$

$\frac{dN}{dt} = \mu(\cdot)N$

F value = concept of destruction (minutes) of a microorganism at 121,18°C (250°F)

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Dublin • Ireland • 12 - 15 September 2011