

The Use of Omic Biomarkers to Track the Survival of *Bacillus weihenstephanensis* KBAB4 Throughout Acid Inactivation

IAFP European Symposium 2011

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Life Sciences

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Foreword

-Microorganisms and Food industry-

- *Bacillus cereus* group

B. cereus group (closely related species)

- ✓ Food-borne pathogen
 - emetic syndrome
 - diarrheic syndrome

- ✓ Food spoilage
 - Dairy industry
 - RTE ...

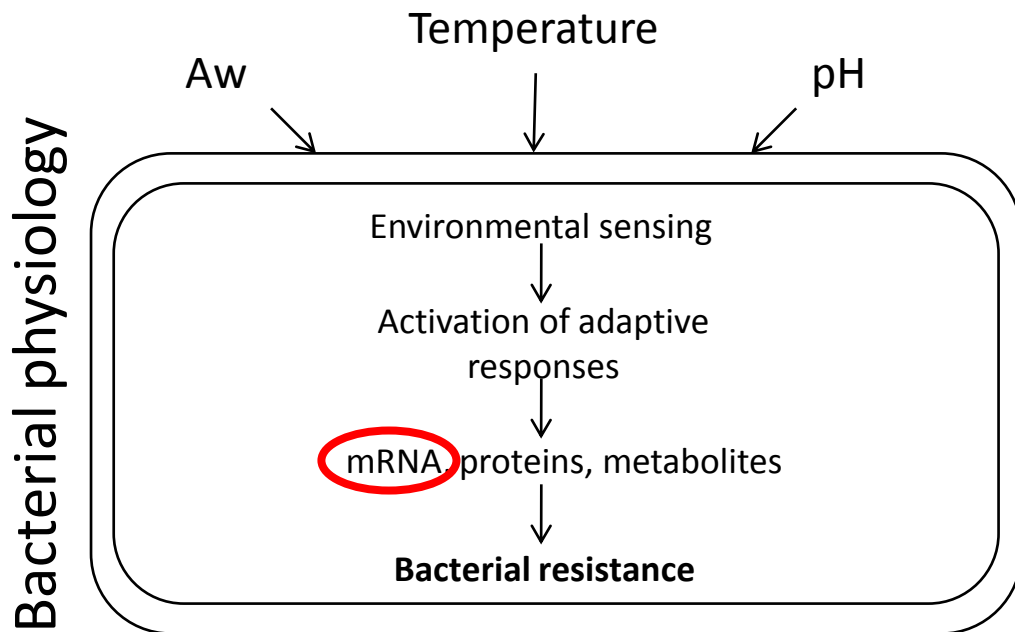
B. weihenstephanensis psychrotolerant species (group VI)

- Food industry



Inactivation or prevention of growth microorganisms using preservation techniques
Mildly stressed cells can be more resistant

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



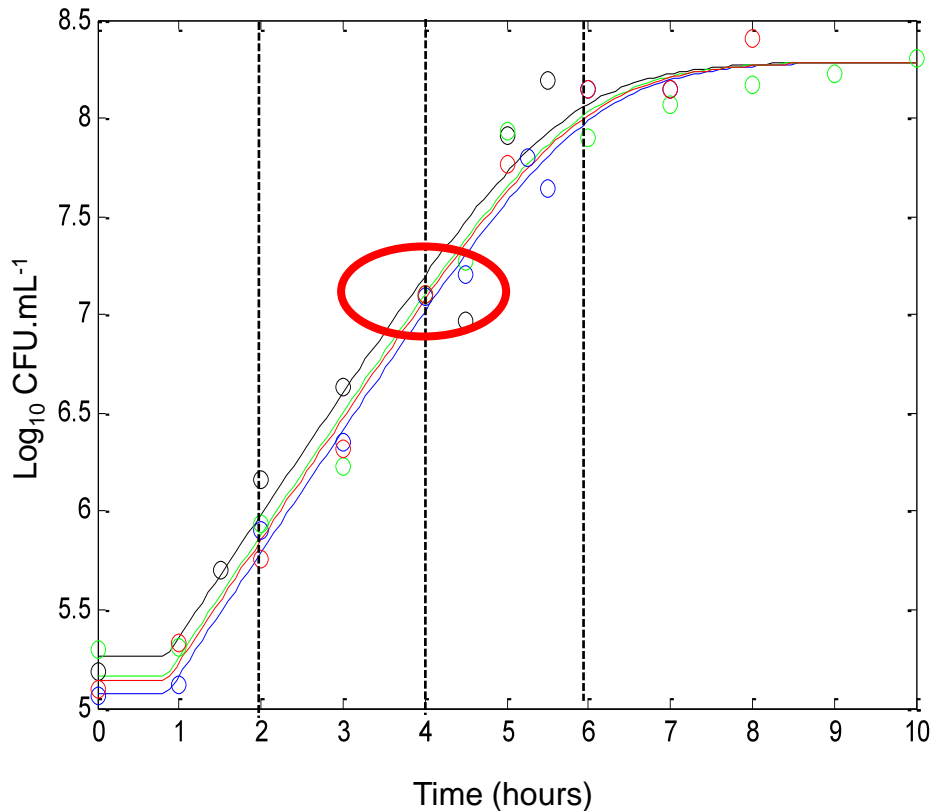
Biomarkers:

- ✓ Characteristic
- ✓ **Objectively measured**
(with satisfactory accuracy and reproducibility)
- ✓ Evaluated **as an indicator**
- ✓ Biologic processes

(Atkinson *et al.*, 2001).

Determination of acid resistance

B. weihenstephanensis KBAB4 growth kinetic
(BHI, 100 rpm, 30°C)



Use of standardized protocol

Reproducible physiological states

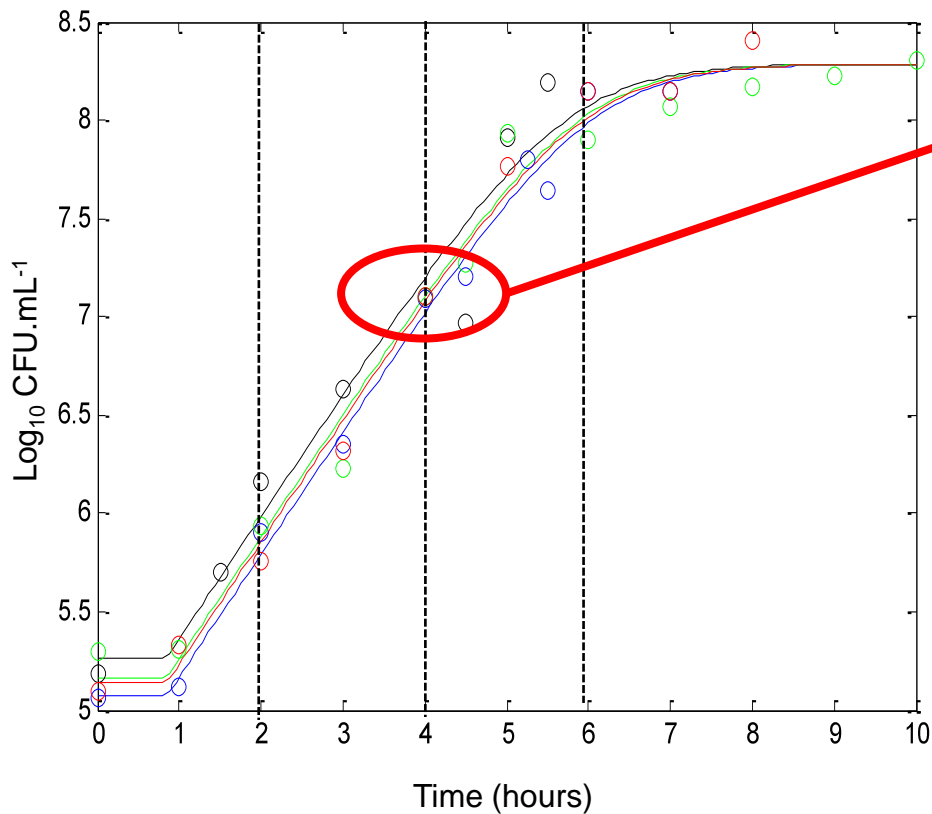
Define by growth conditions

- Beginning of exponentially phase
- Mid exponentially phase
- End exponentially phase

Determination of acid resistance

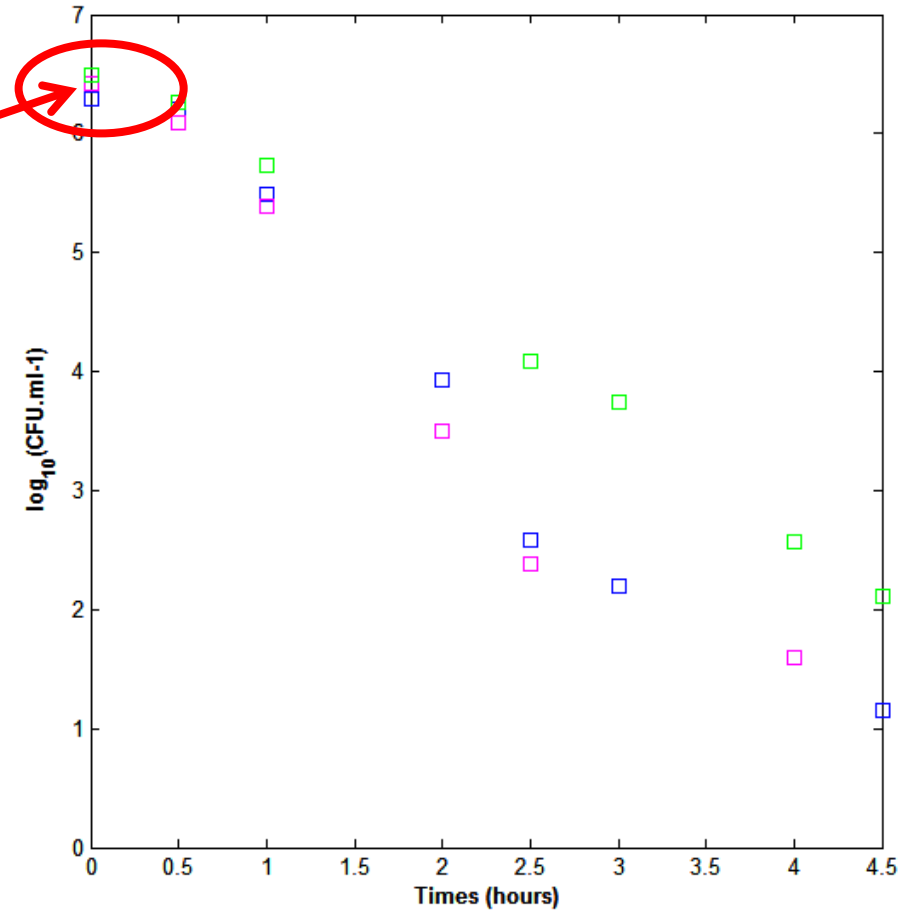
Reproducible physiological states

B. weihenstephanensis KBAB4 growth kinetic
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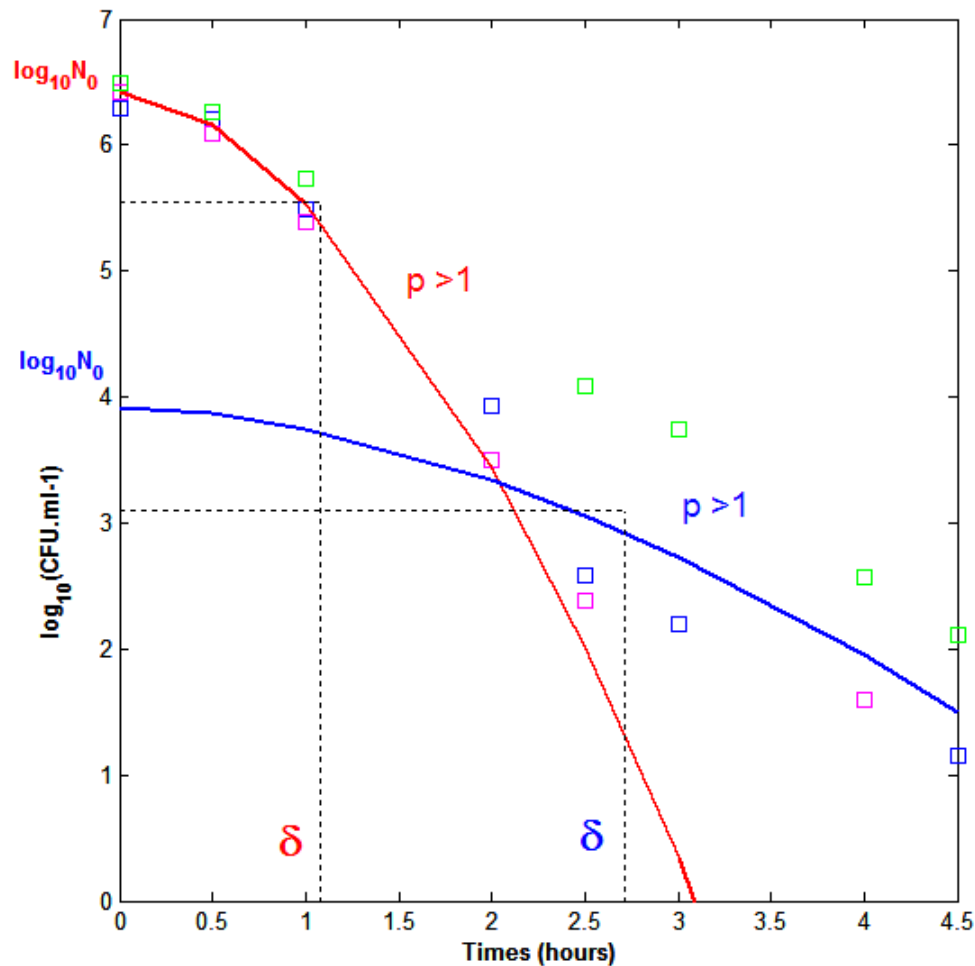
Acid stress pH 4.6

B. weihenstephanensis KBAB4 inactivation kinetic
(BHI, 100 rpm, 30°C, pH 4.6)



Determination of acid resistance

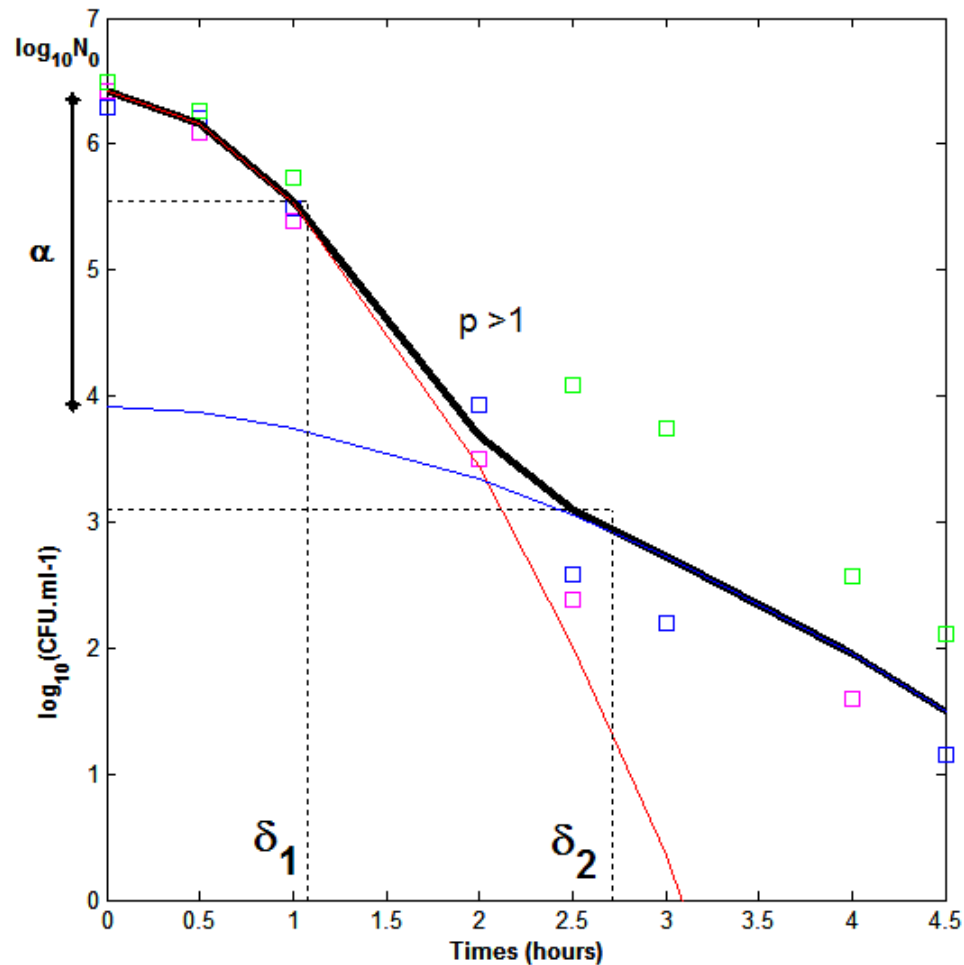
B. weihenstephanensis KBAB4 inactivation kinetic
(BHI, 100 rpm, 30°C, pH 4.6)



- ✓ Two sub-populations
- ✓ **Weibull** survival curve
 - Initial population N_0
 - First decimal decrease δ
 - Shape parameter p

Determination of acid resistance

B. weihenstephanensis KBAB4 inactivation kinetic
(BHI, 100 rpm, 30°C, pH 4.6)



✓ Two sub-populations

✓ **Mixed Weibull model**

- Initial population N_0
- First decimal decrease δ of the two sub-populations
- Shape parameter p
- Population discrimination α

Determination of acid resistance

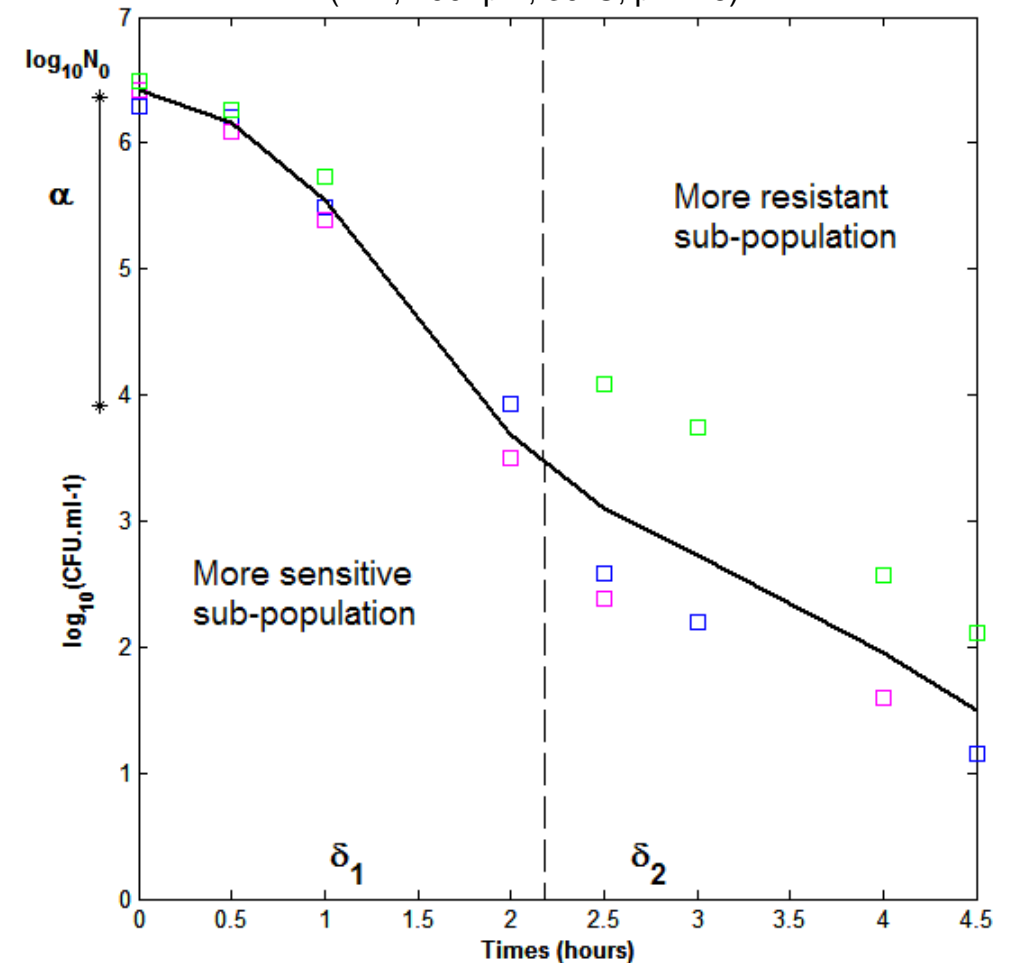
Mixed Weibull model parameters

Name parameters	Estimated value
$\text{Log}_{10}(N_0)$	6.40
δ_1 (hours)	1.10
δ_2 (hours)	2.95
α	2.56
ρ	1.85

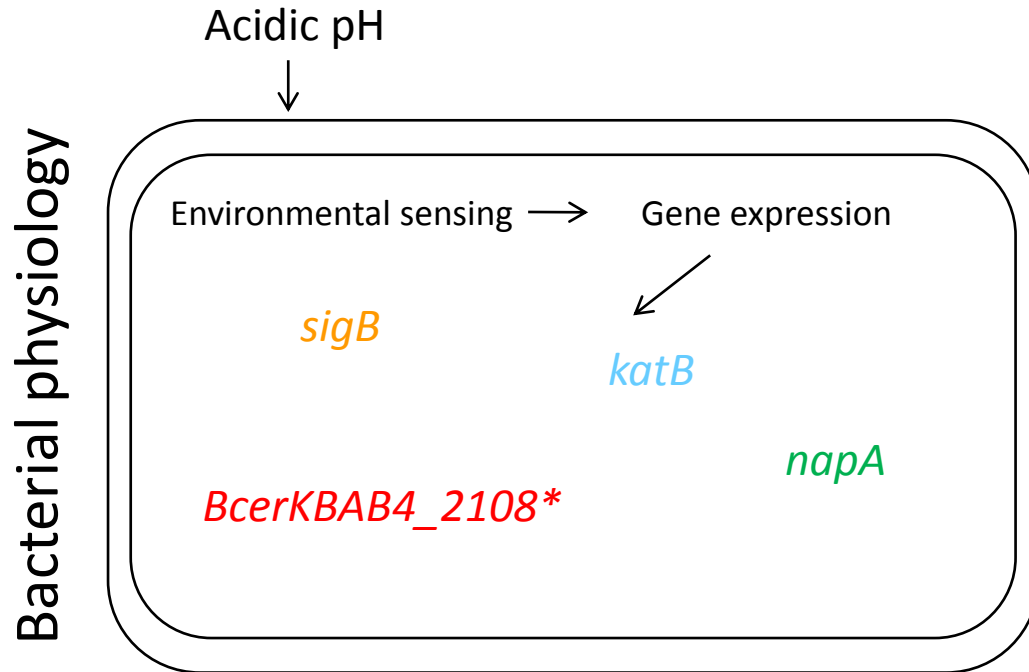
Two sub-populations:
 One more sensitive than the other

Acid stress pH 4.6

B. weihenstephanensis KBAB4 inactivation kinetic
 (BHI, 100 rpm, 30°C, pH 4.6)



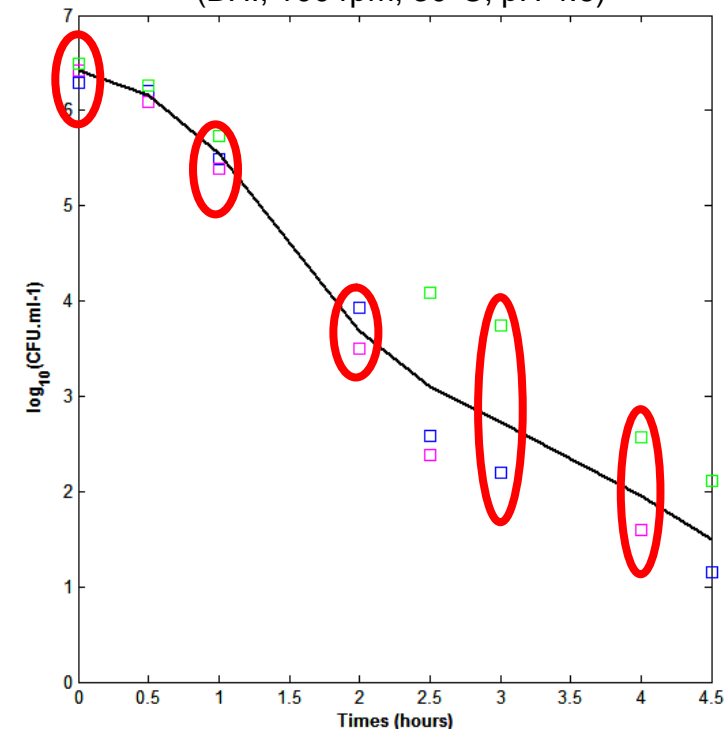
Determination of potential Biomarkers



Gene expression is normalized using 3 housekeeping genes (*tuf*, *sigA*, *gyrA*) yielding satisfactory M value (CFX manager, BIORAD) . For all inactivation kinetics, normalized gene expression is indicated after subtraction of the Unstress control

Acid stress pH 4.6

B. weihenstephanensis KBAB4 inactivation kinetic
(BHI, 100 rpm, 30°C, pH 4.6)



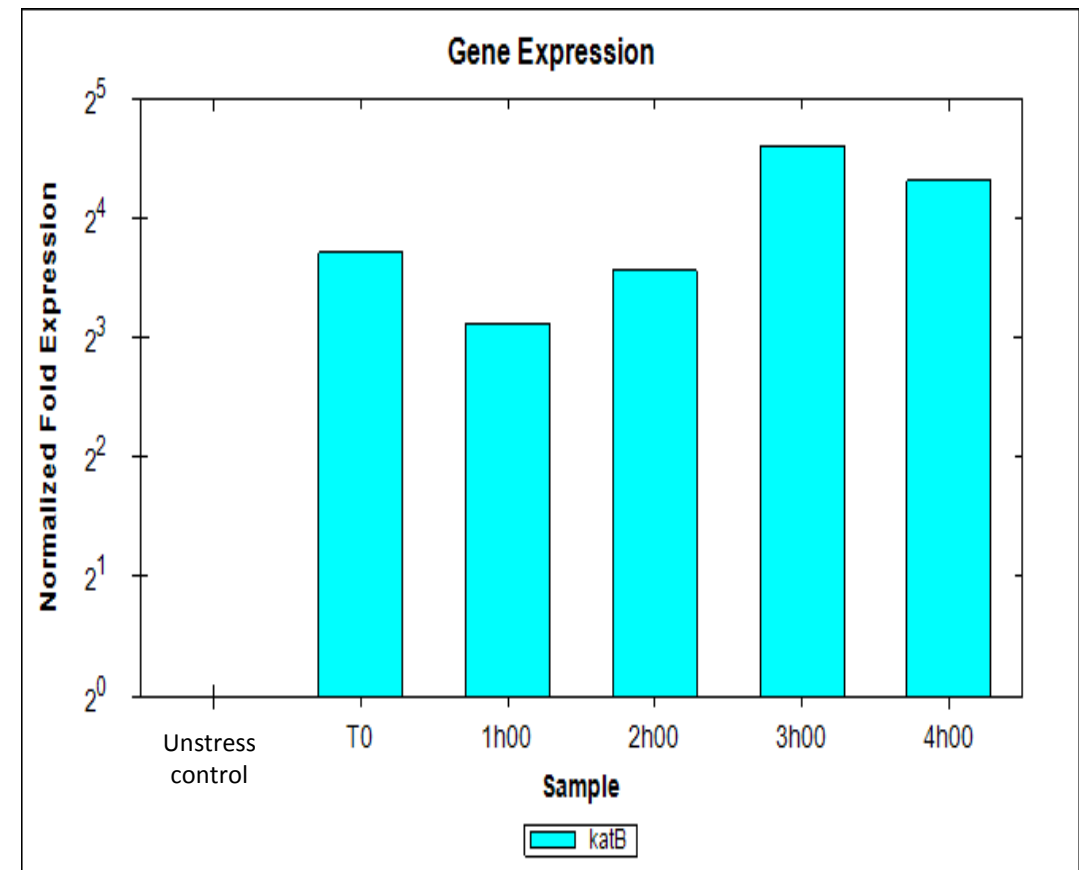
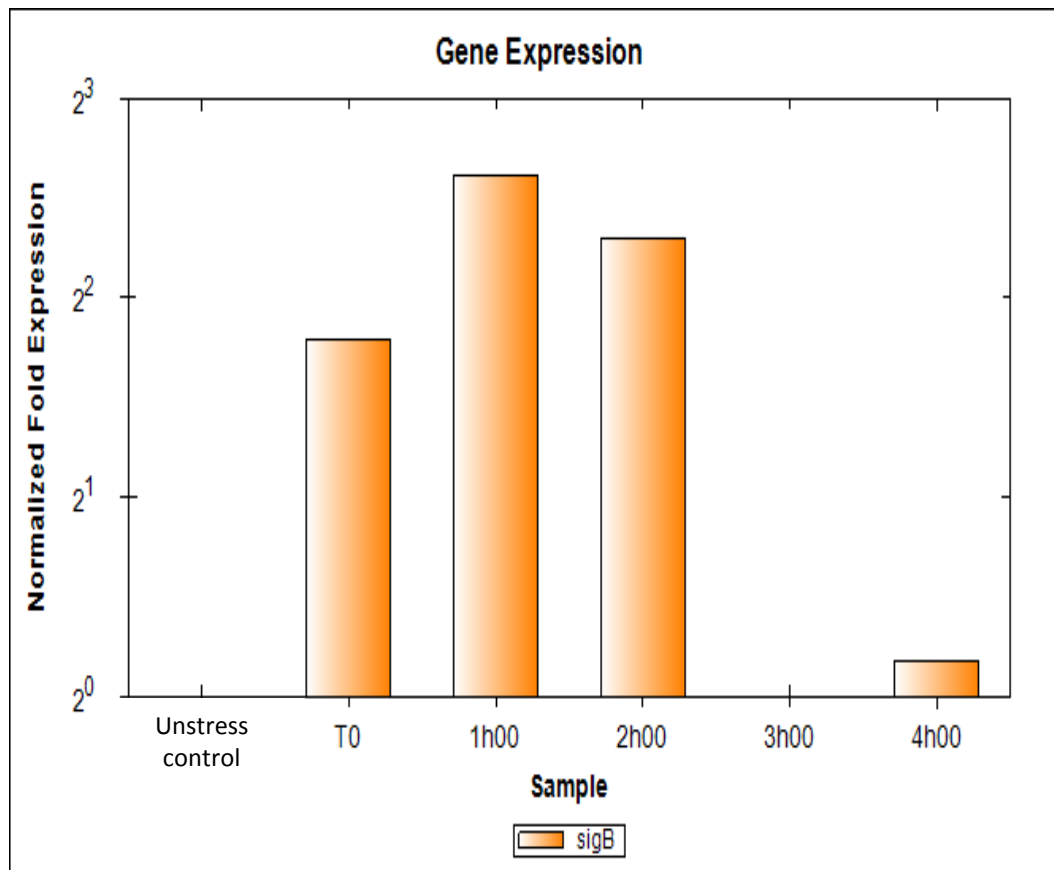
Gene expression :

- RNA extraction
- Reverse transcription
- Quantitative PCR (SybrGreen)

Determination of potential Biomarkers -Genes expression-

Regulators*

Enzymes*



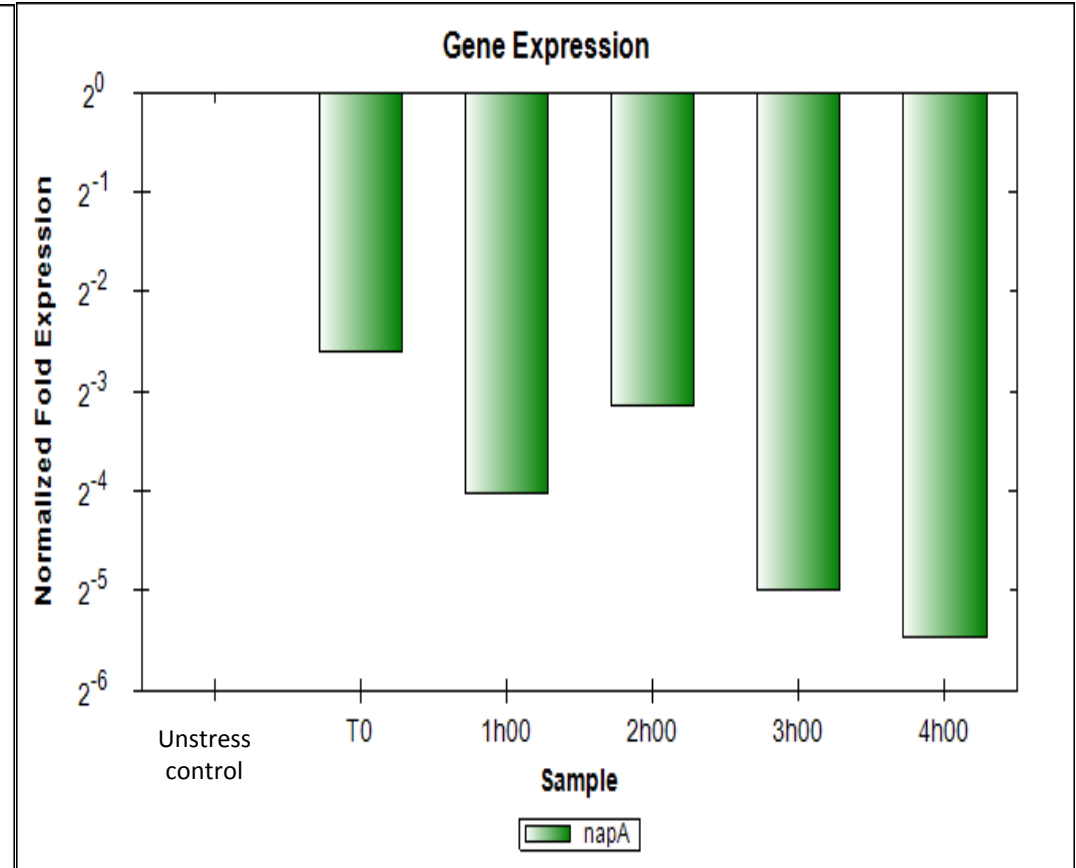
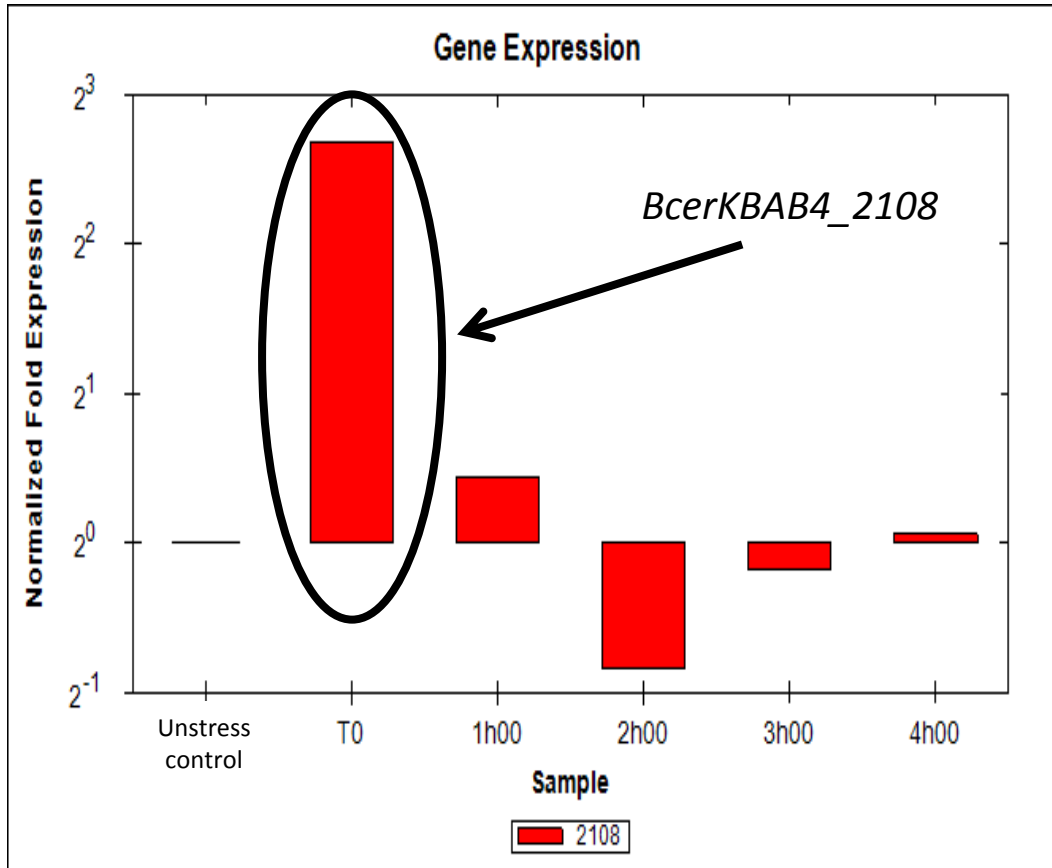
* Genes encoding for

Determination of potential Biomarkers -Genes expression-

Enzymes already used as biomarkers*

(Den Besten *et al.*, 2010)

H⁺ antiporter & nitrogen metabolism*



* Genes encoding for

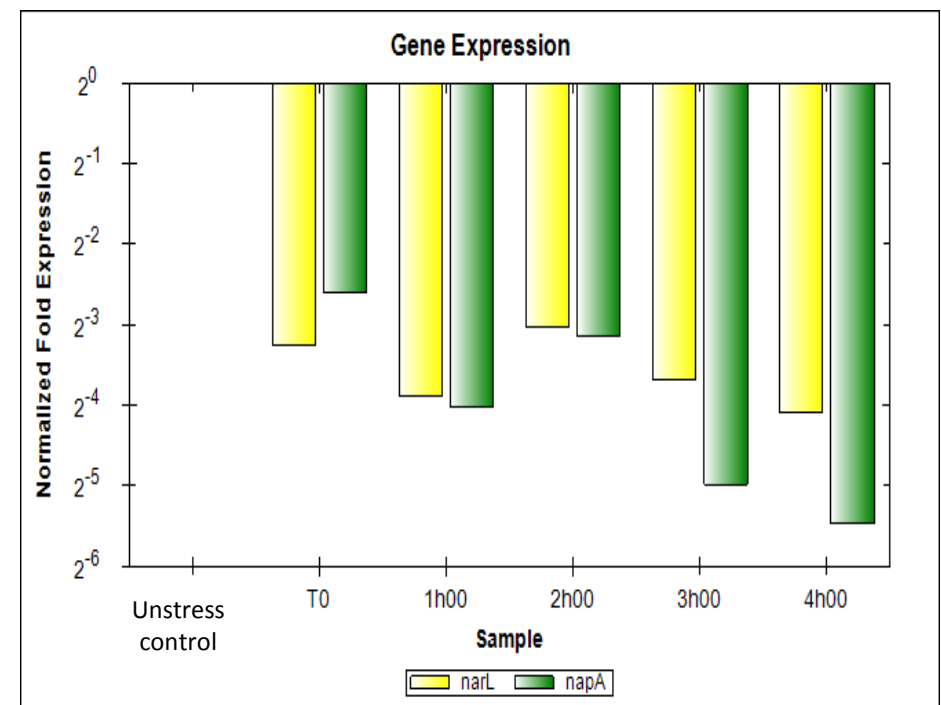
Determination of potential Biomarkers -Cells viability-

Cells viability biomarkers are defined using various criteria (Kort *et al*, 2008):

- High expression levels in living bacteria
- Relatively low expression levels in stressed bacteria
- Genes widely conserved

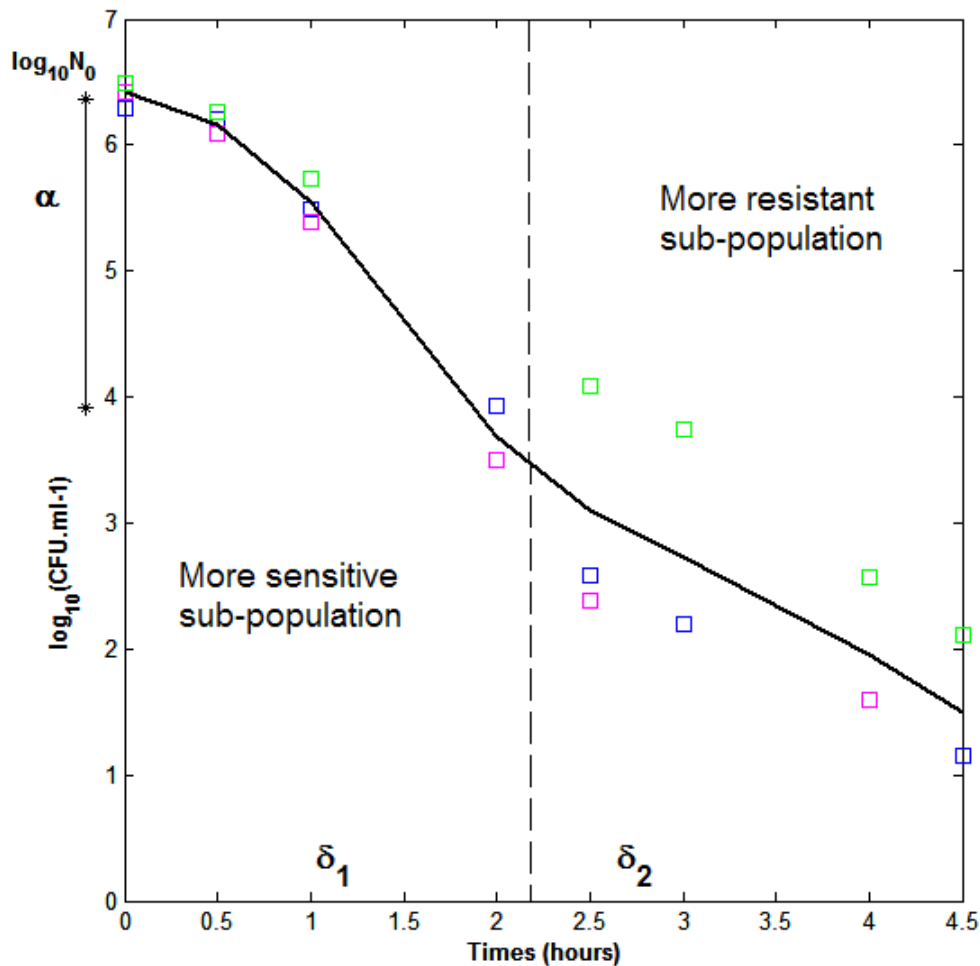
Two genes down-regulated throughout the acid inactivation (normalized compared:

- *narL* (nitrate reductase)
- *napA* (proton antiporter)



Determination of potential Biomarkers -Cells resistance-

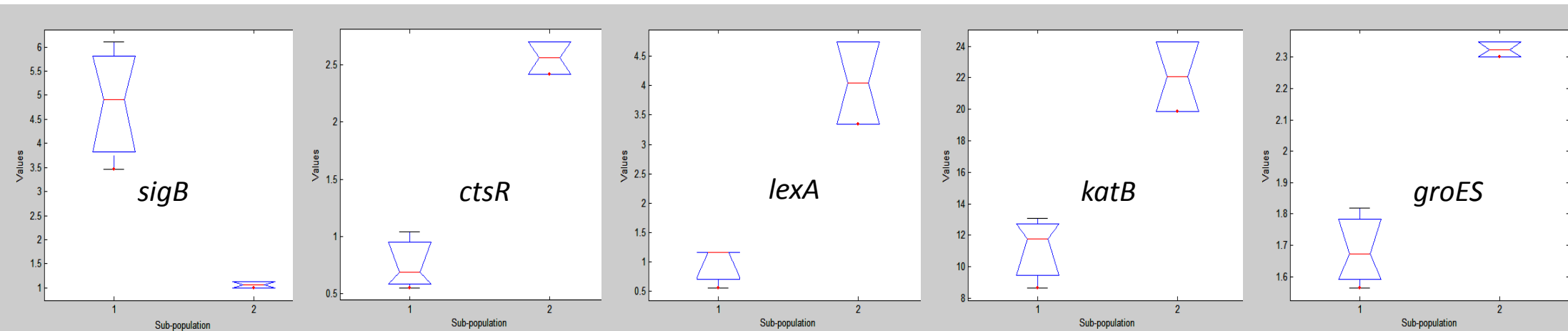
B. weihenstephanensis KBAB4 inactivation kinetic
(BHI, 100 rpm, 30°C, pH 4.6)



Is there is any difference
between the gene expression
of the two sub-populations ?

ANOVA one way for each genes expression

Determination of potential Biomarkers -Cells resistance-



Five genes have **significant variation** between the two sub-population (*i.e.* $p < 0.05$)

- *sigB* (General stress response)
- *ctsR* (Transcriptional regulator)
- *lexA* (SOS response transcriptional repressor)
- *katB* (Catalase)
- *groES* (Chaperone protein)

And may be used as resistant Biomarkers

Identification of molecular biomarkers that can predict the acid stress resistance of *B. weihenstephanensis* KBAB4

- Better understanding of the bacterial inactivation
- Determination of the physiological state
- Promising results to determine both viability or resistance biomarkers
- Further investigations are necessary
 - ✓ With other physiological states (mild stress adaptation acid, cold, salt and oxidative stress)
 - ✓ Using others genes as potential biomarkers

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