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Mapping the physiological states of *Carnobacterium maltaromaticum* obtained by applying stressful fermentation conditions

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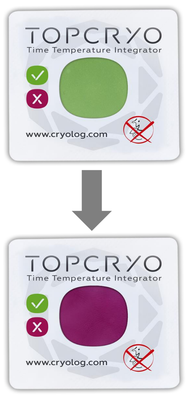
Context & Aim

Time-temperature Integrators (TTIs) are smart-labels that relay the time-temperature history of the food they are attached to[1]. They are used for detecting a break of the cold chain. Biological TTIs contain lactic acid bacteria (LAB) which growth induces a pH drop and a color shift, thus indicating that the food is no longer fit for consumption[2]. There is a need to improve their performances and widen the shelf-life range they cover (currently 30 h to 8 days). It has been proven that fermentation conditions can modulate the physiological state of LAB[3][4]. The main hypothesis that has lead to this work is that changing the physiological state of LAB during fermentation impacts the acidification activity of the harvested cells, within the TTI label medium.



Aim of this work: **Map out the possible acidification activities of freeze-thawed LAB of industrial interest, by changing fermentations parameters (temperature, pH and harvest time)**

Methods

Studied Factor	Range
Temperature (°C)	20 – 37
pH	6 – 9.5
Harvest time (h)	t = 0 when max acidification rate is reached

Inoculum
C. maltaromaticum CNCM I-3298

Fermentation
(MN medium – 150 rpm)

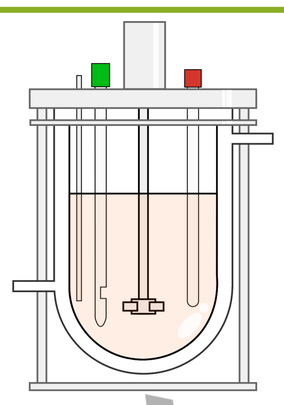
Concentration
(2 635g – 10 min – 4°C)
Protection
(trehalose solution)
Freezing
(-80°C – 3 °C.min^{-1})

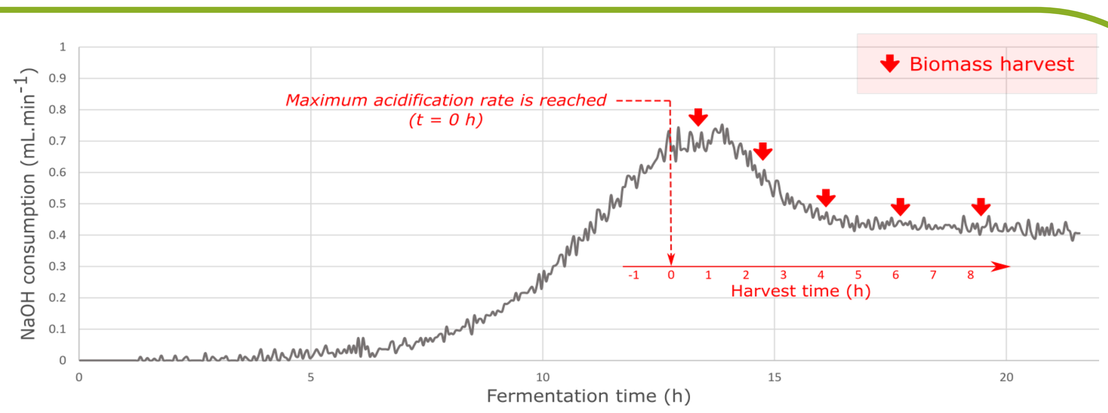
Freeze-thawing
of bacterial concentrates

(regulated pH)
- **Acidification rate** of fermentation medium (NaOH 5N consumption)
- **Biomass** (Optical density and dry-weight)
- HPLC → **Trehalose** consumption → **Lactic acid** production

(unregulated pH)
- **Acidification activity** (TopCryo® like medium – 30 °C) → dtpH1.5
- **Cultivability** measurement (Plate count)

Physiological state : cell growth and acidification properties during fermentation – – ? – – → **Cultivability and acidification activity of concentrates**

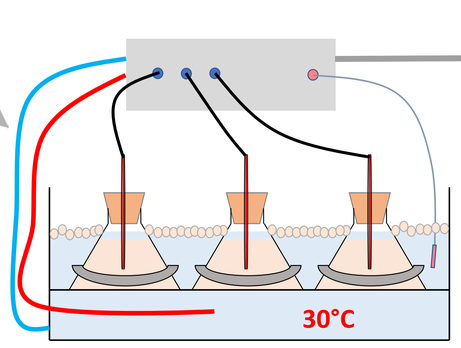


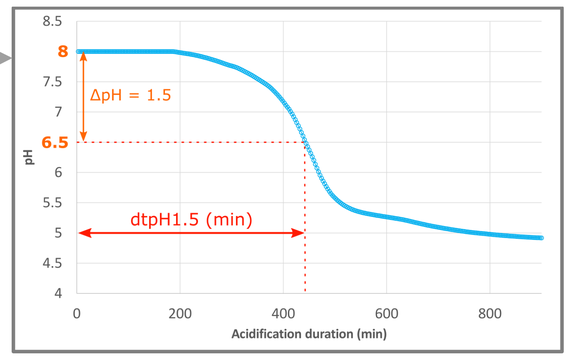


Harvests were done at increasing times after the maximum acidification rate of the fermentation medium was reached.

dtpH1.5 ↗ → Acidification activity ↘

Cinac® system → **Acidification activity**





The acidification activity of the freeze-thawed bacterial cells was done in triplicate, for each harvest time.

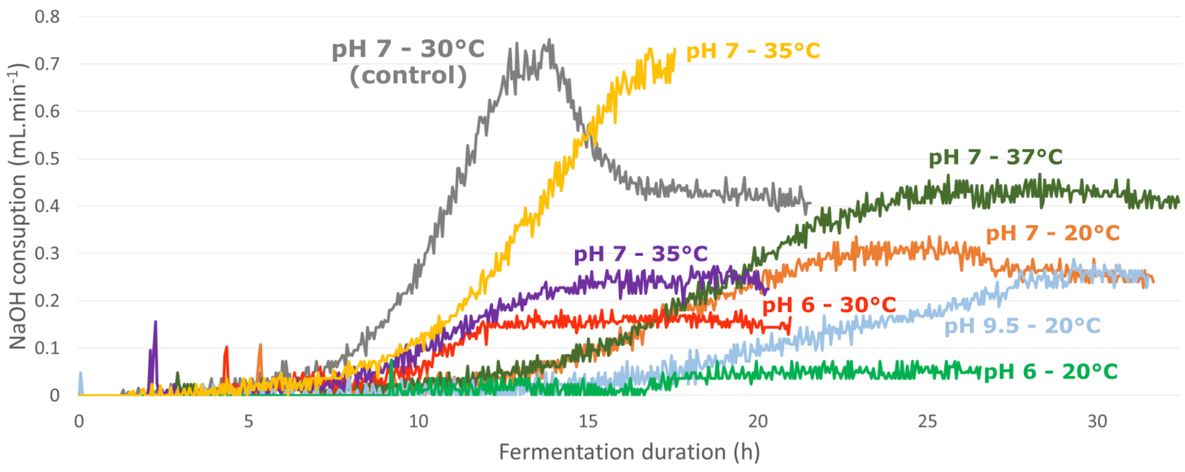
Results & discussion

✓ **Effect of pH and temperature on physiological state during fermentation:**

- → pH 7.0, 30 °C ⇨ best parameters for maximum cell growth and acidification rate;
- → pH ≠ 7.0 ⇨ maximum acidification rate during fermentation ↘;
- → T ≠ 30 °C ⇨ maximum acidification rate during fermentation ↘ and fermentation duration ↗.

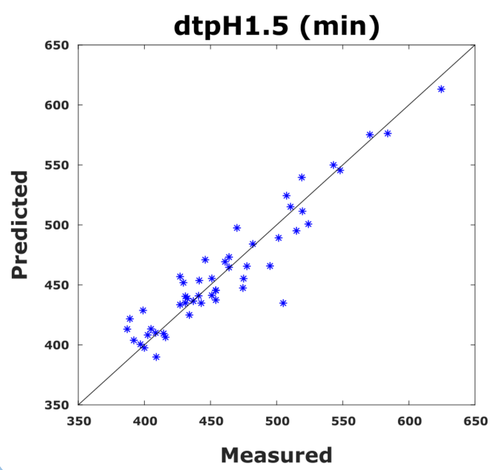
✓ **Effect of pH, temperature and harvest time on dtpH1.5:**

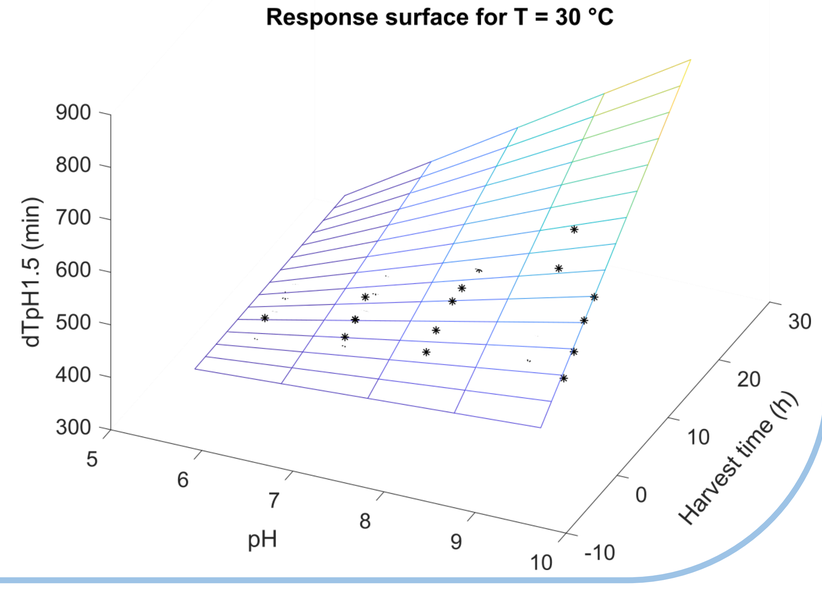
- → slight influence of temperature: no technological interest;
- → pH 6 ⇨ dtpH1.5 ↘ by 55 min;
- → pH 9.5 ⇨ dtpH1.5 ↗ by 110 min;
- → high pH values emphasized the detrimental effects of harvest time.



✓ **Regression model:** $dtpH1.5 = 310.54 - 18.69 \times ht + 0.1 \times T \times pH + 3.31 \times pH \times ht - 0.09 \times T^2$

T : temperature (°C) *ht : Harvest time (h)*





Conclusion & Prospects

☑ This research identified **pH and harvest time** to be the most efficient fermentation stressors for the production of *C. maltaromaticum* concentrates exhibiting extreme physiological states.

To better understand the mechanisms underlying the bacterial stress responses, two main research axes can be pursued:

→ Characterizing the **cell's membrane composition and organization** to identify it's role in physiological state;

→ Setting up **intracellular pH** measurements by flow cytometry to investigate the pH effect.

[1] **Taoukis and Labuza.** « Applicability of time-temperature indicators as shelf life monitors of food products ». *Journal of Food Science* 54, nº 4 (1989): 783–788.

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[3] **Fonseca, F., C. Béal, and G. Corrieu.** « Operating Conditions That Affect the Resistance of Lactic Acid Bacteria to Freezing and Frozen Storage ». *Cryobiology* 43, nº 3 (2001): 189-98.

[4] **Rault, A., M. Bouix, and C. Béal.** « Dynamic Analysis of Lactobacillus Delbrueckii Subsp. Bulgaricus CFL1 Physiological Characteristics during Fermentation ». *Applied Microbiology and Biotechnology* 81, nº 3 (2008): 559-70.