Fermentation of Chlorella vulgaris, a microalga used in human food, by 89 strains of lactic bacteria

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CONTEXT & AIM

- ✓ Microalgua are photosynthetic unicellular organisms rich in nutriments, constituting a biomass with high potential for food applications.
- ✓ Their production is energy consuming with a high risk of alteration by spoilage flora during the process. Fermentation, an ancestral preservation process, offers an alternative to current processes.
- ✓ By degrading the sugars naturally present in the matrix, lactic acid bacteria (LAB) produce organic acids, thus lowering the pH, which helps to inhibit pathogenic and spoilage flora when the pH reaches values around 4.6.
- ✓ **In this study, we screened the ability of 89 LAB strains (10 species) to ferment *Chlorella vulgaris*, microalgae approved for human consumption.**

STRATEGY

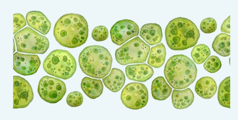
Table 1 : Number of tested LAB strains by species

Species	Number of strains
Levilactobacillus brevis	4
Pediococcus pentosaceus	4
Lactiplantibacillus pentosus	5
Lactiplantibacillus paraplantarum	3
Lactiplantibacillus plantarum	14
Lacticaseibacillus paracasei	5
Loigolactobacillus coryniformis	2
Leuconostoc mesenteroides cremoris	2
Fructilactobacillus fructivorans	1
Lactococcus lactis	7
TOTAL	89

Strains provided from the International Centre of Microbial Resources dedicated to Food Bacteria (CIRM-BIA). https://collection-cirmbia.fr/

SCREENING OF 89 STRAINS ON THEIR ABILITY TO FERMENT *CHLORELLA VULGARIS*

1 tested LAB strain
C_i = 6 log_{10} CFU/ml



Chlorella vulgaris
C_i = 50 mg/ml

Incubation at 30°C
Aerobiosis
12 h, 24 h and 36 h

- ✓ Microscopic observations
- ✓ pH measurement
- ✓ LAB enumeration
- ✓ Contamination flora enumeration and 16S identification

Selection of 3 strains

OPTIMIZATION OF FERMENTATION
(Ability to reach a target pH=4.5)

Chlorella vulgaris
C_i = 50 mg/ml

+

1 selected LAB strain
C_i = 6 log_{10} CFU/ml

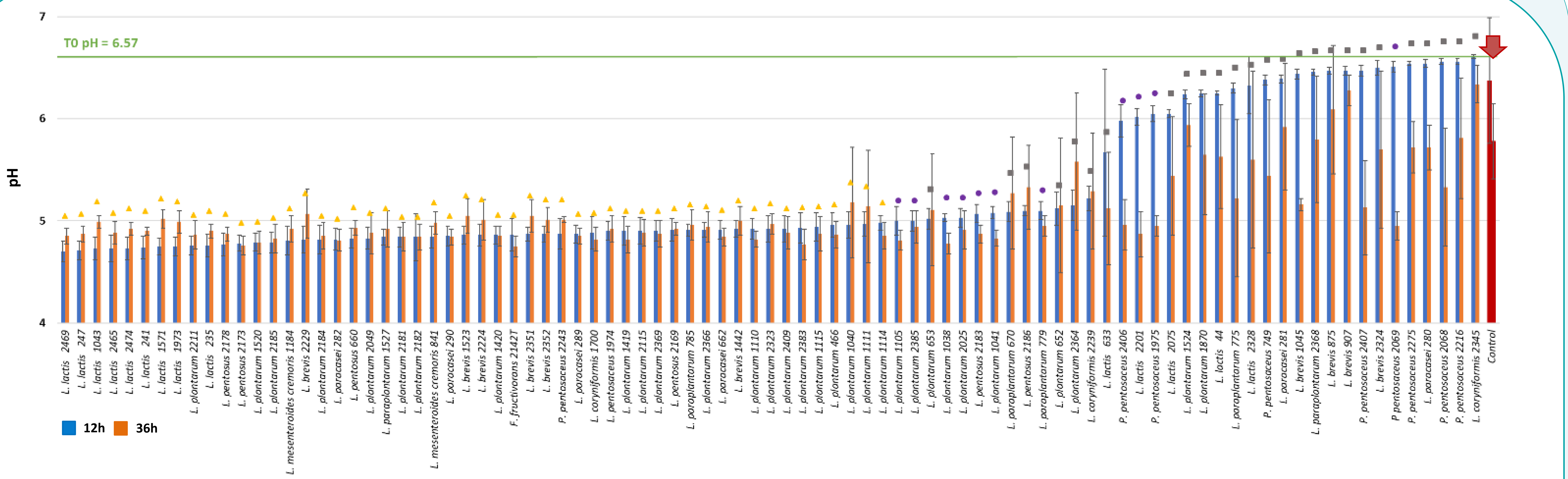
Glucose
(C_f=0.5 g/L; 1g/L; 2g/L)
and/or
Yeast Extract
(C_f=5 g/L)

Incubation at 30°C
Aerobiosis, 24 h

- ✓ Microscopic observations
- ✓ pH measurement
- ✓ LAB enumeration

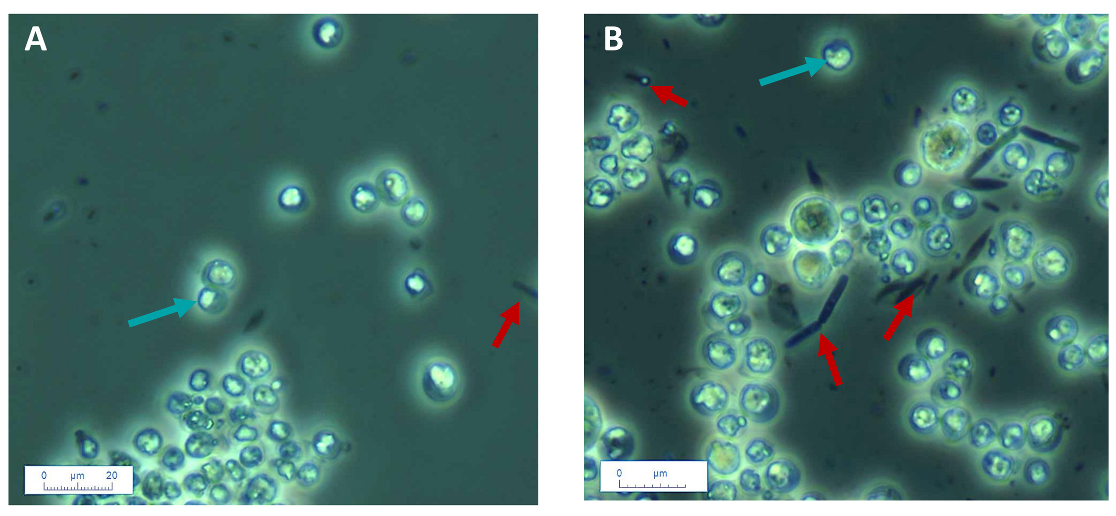
RESULTS

ABILITY OF 89 LAB STRAINS TO FERMENT *CHLORELLA VULGARIS*



- ➢ A pH decrease was observed for the majority of the strains (72%) at 12h. After 12h of fermentation, the lowest pH reached 4.7 with the strain *Lactococcus lactis* CIRM-BIA2469.
- ➢ The fermentation profiles of the 89 strains were divided into three « acidification groups »:
  - group of strains which finished acidifying at 12h (60% of the strains); ▲
  - group of strains which finished acidifying at 36h (12% of the strains); ●
  - group of non-acidifying strains with no acidification at 36h (28% of the strains). ■
- ➢ ***Lactococcus lactis, Lactiplantibacillus plantarum*** and ***Lactiplantibacillus pentosus*** were the most acidifying species.
- ➢ A pH decrease was observed for the unfermented microalgal biomass used as the control, 5.78±0.37 (red arrow) suggesting the development of a native spoilage flora.

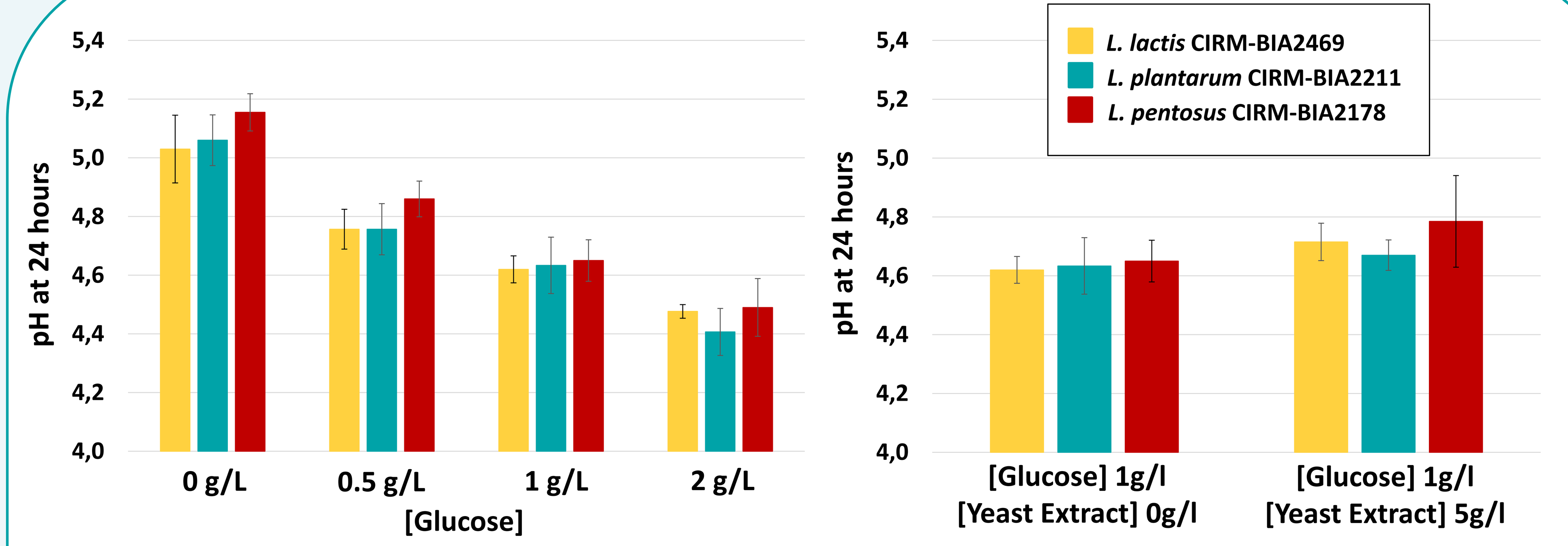
IDENTIFICATION OF NATIVE SPOILAGE FLORA



Microscopic observations (X100) of a non-inoculated suspension of *C. vulgaris* at T0 (A) and after 24 hours (B) of incubation at 30°C. Blue arrows indicate *Chlorella vulgaris* and red arrow shows native spoilage flora.

- ➢ A native flora was present in the suspension of *Chlorella vulgaris* around 3 log_{10} CFU/ml at T0, enumerated on BHI-YE.
- ➢ The spoilage flora was identified by sequencing of DNAr16S as ***Bacillus sp.***
- ➢ After 24h of incubation at 30°C, the concentration of the native flora reached 8 log_{10} CFU/ml in the non-inoculated suspension.
- ➢ When the algal suspension was inoculated with LAB, the spoilage flora was not enumerated on BHI-YE (data not shown).

OPTIMIZATION OF FERMENTATION



- ➢ The optimization was performed on the three best strains from the screening belonging to three different species (*L. lactis*, *L. plantarum* and *L. pentosus*).
- ➢ Addition of glucose enhanced the acidification rate for the three strains used. The effect was dependent on the concentration of glucose. Addition **of 2g of glucose** allowed to reach the targeted **pH of 4.5** for all strains.
- ➢ The addition of yeast extract combined with glucose did not increase the acidification rate compared to glucose alone, whatever the strains used.

CONCLUSION & PERSPECTIVES

- ✓ **We confirmed the ability of LAB to ferment a freeze-dried *Chlorella vulgaris* biomass. To our knowledge, this is the first study of microalgae fermentation by such a large number of LAB strains.**
- ✓ **The LAB were able to inhibit the native spoilage flora, identified as the genus *Bacillus sp.*.**
- ✓ **The addition of glucose allowed to optimize the acidification rate with a targeted pH around 4.5 after 24h of fermentation.**
- ✓ **Work is in progress to extend the screening to other freeze-dried microalgae species as well as to fresh microalgae supensions.**

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