Growth study of an *E.coli* GMO strain incapable of catabolizing lactic acid in mixtures of organic acids and simple sugars

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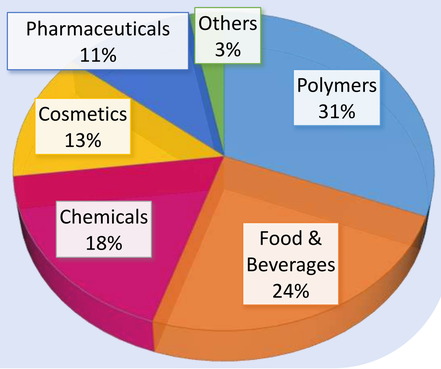
INTRODUCTION

Background

- Lactic acid (LA) is a biomolecule that finds several industrial and biomedical uses (e.g. food, cosmetics, textiles, pharmaceuticals, etc.)
- LA is mainly produced through a fermentation process
- To achieve high purity lactic acid at low cost remains a challenge

Lactic acid

$C_3H_6O_3$



Objective

This study assesses the growth of an engineered *E.coli W3110* strain on different synthetic substrates. The strain is genetically modified to be incapable of utilizing Lactic Acid (LA), but capable to selectively consume other organic acids and simple sugars.

Approach

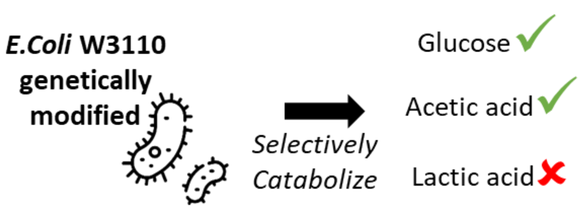


Figure 1. Representation of the GMO *E.coli* strain capabilities

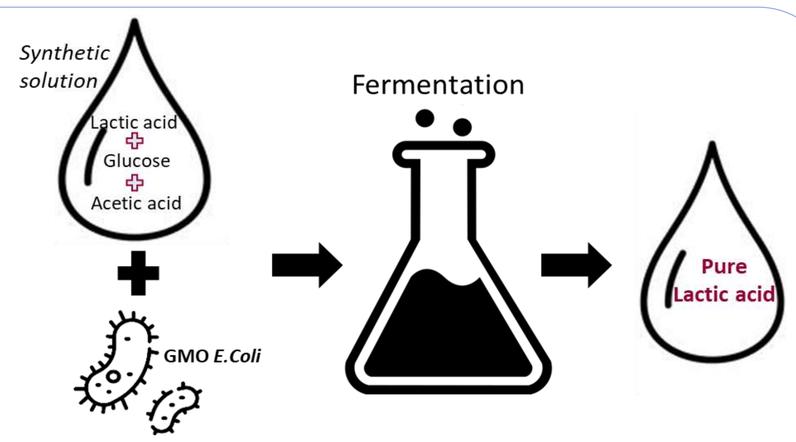


Figure 2. Schematic concept representation of the current study

EXPERIMENTAL DESCRIPTION

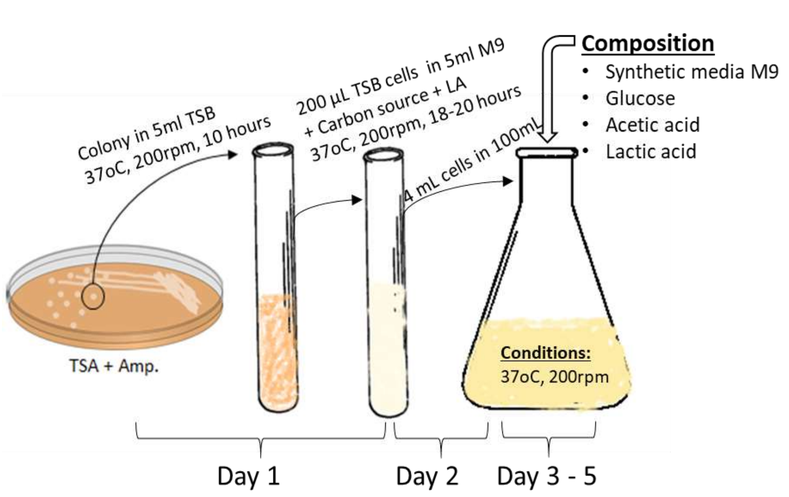


Figure 3. Experimental process description

Main goal

➢ Study the effect of different conditions on cells' growth

Objectives

1. Study the effect of the 2nd pre-culture step
2. Study the effect of carbon sources concentration (Glucose and Acetic Acid – AA)
3. Study the effect of Lactic acid (LA) concentration

General conditions

- 100 mL batch fermentation in shake flasks
- Triplicates for each tested condition
- Sampling every 24hrs
- Negative control flask for every test
- Glucose:AA→ 1:1

Measured parameters

- Optical density (OD600)
- pH measurement
- Total Nitrogen
- Total Organic Carbon (TOC)
- Glucose and AA conc.
- LA conc.

RESULTS AND DISCUSSION

1. Effect of 2nd Pre-culture step:

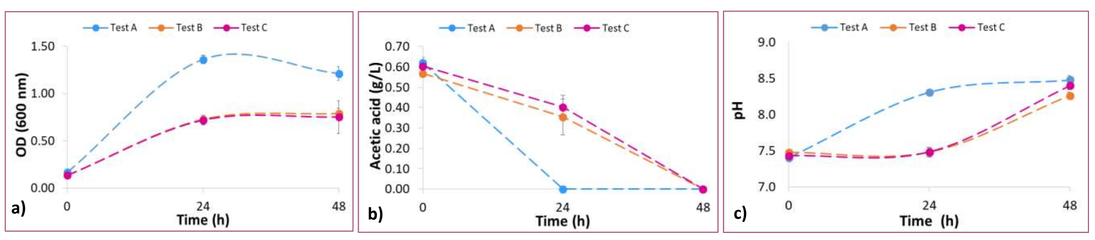


Figure 4. Results on the effect of a 2nd pre-culture step and its carbon source composition. **Test A**: Test without the 2nd pre-culture step; **Test B**: 0.5 g/L glucose + 0.5 g/L AA as carbon source in the 2nd pre-culture step; **Test C**: 1.0 g/L glucose + 1.0 g/L AA as carbon source in the 2nd pre-culture step. **a)** Cells' growth measurements (OD600); **b)** AA consumption as a function of time; **c)** Culture pH as a function of time.

- Substrate composition in the 2nd pre-culture step does not seem to affect the main fermentation process
- Batch fermentation by inoculating directly from tryptic soy broth culture results in better growth characteristics since TSB is rich in nutrients media compared to M9

2. Lactic acid concentration effect:

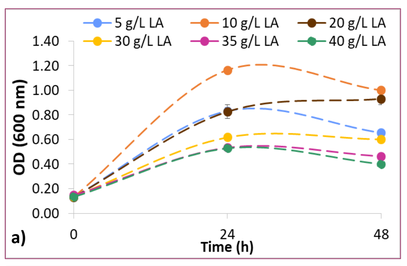


Figure 5. Results from batch fermentation tests to assess the effect of LA concentration (5 to 40 g/L LA) on:
a) Cells' growth measurements (OD600);
b) The AA consumption profile;
c) Lactic acid concentration profile for different initial LA concentration.

- **Glucose is consumed within 24 h for all the tested cases (data not shown)**
- **The initial LA concentration seems to affect the fermentation process**
  - The growth of *E.coli* strain is inhibited as the LA concentration increases (Figure 6a)
  - Acetic acid consumption is limited as the LA concentration increases (Figure 6b)
  - For LA concentration higher than 30 g/L, AA is not catabolized while for 20 g/L the AA consumption is 24hrs delayed
- **LA concentration remains constant during the fermentation (Figure 6c)**

3. Glucose/Acetic acid conc.:

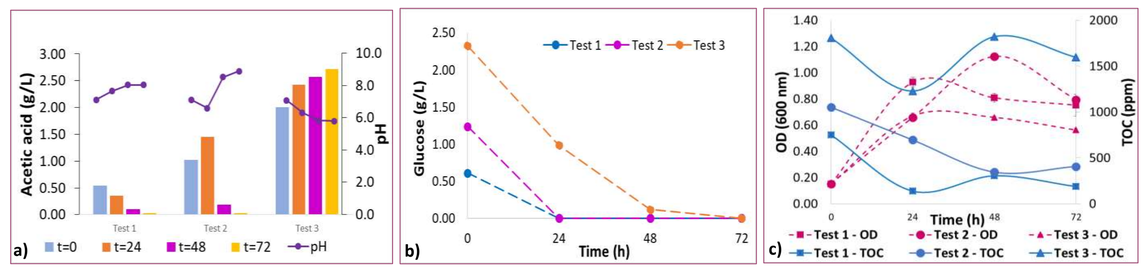


Figure 6. Results from batch fermentation tests with different concentrations of carbon sources. **Test 1**: 0.5 g/L glucose + 0.5 g/L AA; **Test 2**: 1.0 g/L glucose + 1.0 g/L AA; **Test 3**: 2.0 g/L glucose + 2.0 g/L AA. **a)** AA concentration temporal profile compared to culture's pH profile; **b)** Glucose consumption as a function of time; **c)** Cells' growth measurements (OD600) compared to TOC concentration of the cultures' medium.

- Due to glucose consumption, AA is excreted in the culture media. The higher the glucose concentration the higher the AA production, which results in pH decrease down to 5.40
- The growth is hindered by the pH drop
- Complete glucose consumption is delayed for 48 h at 2 g/L compared to 0.5 and 1 g/L (initial concentration)
- The lowest TOC concentration of the culture medium is achieved at the end of the exponential growth phase.

CONCLUSIONS

- ✓ The experiments confirm the selective utilization of glucose and AA by the GMO *E.coli* strain
- ✓ LA remains constant in every experiment, but the cells' growth is affected by the high initial LA concentration
- ✓ A 2nd pre-culture step at the same carbon sources as the main culture is preferred as more representative of a scaled-up process in a lab-scale bioreactor
- ✓ The experimental conditions of 0.5:0.5 g/L glucose:AA in the presence of 20 g/L LA is selected for future experiments

ACKNOWLEDGEMENTS

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