Continuous intracellular pH measurement: *Escherichia coli* culture medium pH dependence

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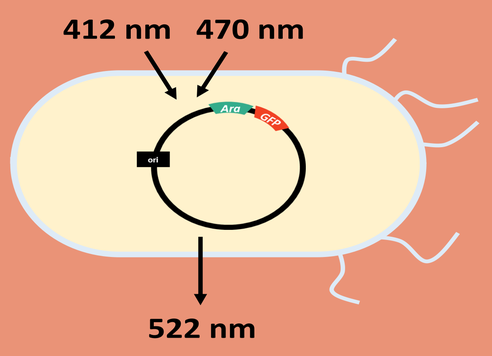
INTRODUCTION

- ***Escherichia coli* (*E. coli*) is the most used host microorganism to high-valuable compounds production in current industry.**
- **Cells pH homeostasis is a crucial factor to grow in different environments maintaining an intracellular pH control, however, to our knowledge, there is not a study about *E. coli* continuous intracellular pH growing in a culture.**

METHODS

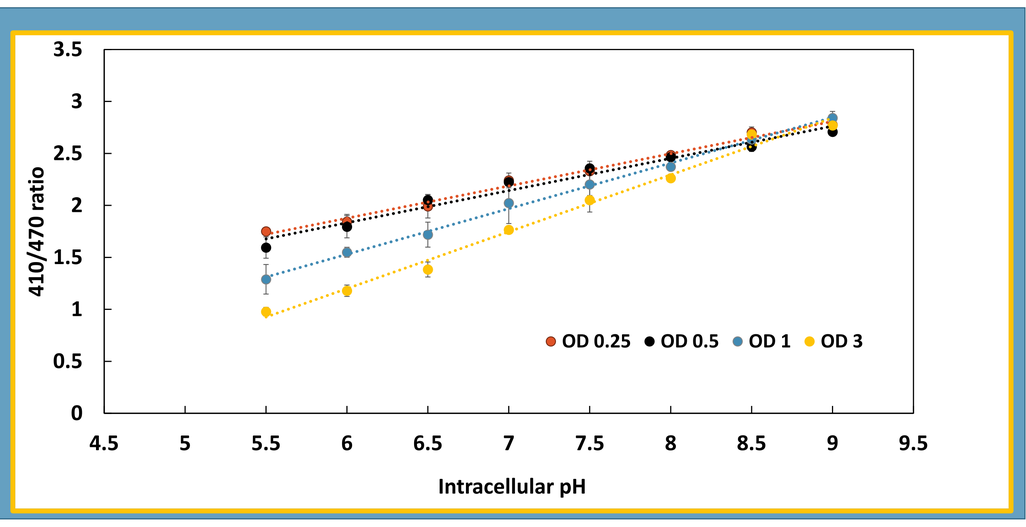
- ***E. coli* BW25113 was grown in minimal medium MM9 or in complex TB7 medium supplemented with glucose 22 mM or glycerol 44 mM as carbon source. Strains were grown at 37 ºC in two different way: on a rotatory shaker at 250 rpm to monitor spectrophotometrically the optical density at 600 nm (OD600) and the extracellular pH, and in a microplate reader (Synergy H1 Hybrid Multi-Mode Reader) to simultaneously monitoring growth and fluorescence.**
- **To determine intracellular pH, the pGFPR01 plasmid, which expresses ratiometric pHluorin, was used. Thus, as pH increased, ratiometric pHluorin showed an increased in excitation at 410 nm and decreased excitation at 470 nm (1). Intracellular pH was determined from the 410/470 fluorescence ratio.**



RESULTS

Method calibration

- **A dependence between the OD at 600 nm and the fluorescence ratio was observed when the pH was lower than 8.**
- **A calibration curve was constructed for different OD (0.25, 0.5, 1, 3).**
- **The method was lineal from pH 5.5 to 9.**



E. coli intracellular pH dependence with carbon source

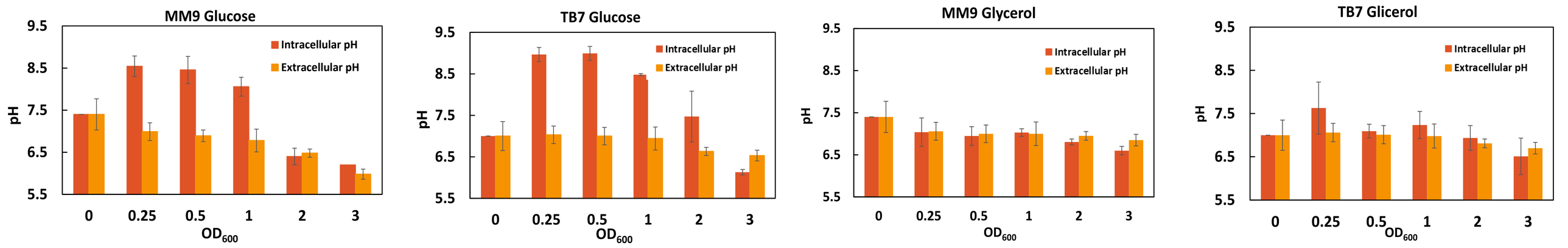
- **In *E. coli* cultures supplemented with glucose, intracellular pH reached values higher than 8.5 during mid-exponential growth phase.**
- **In glycerol cultures, intracellular pH did not increase, showing a slightly decrease to 6.6-6.8 values.**
- **Extracellular pH remained almost constant throughout the culture in those supplemented with glycerol**

Glucose carbon source

Weak intracelular pH homeostasis

Glycerol carbon source

Strong intracelular pH homeostasis



The results show the dependence between the intracellular pH and the carbon source in *E. coli.*

The measurement method developed is a very useful tool for the control of bioprocesses.

REFERENCES

(1) Wilks JC, Slonczewski JL. pH of the cytoplasm and periplasm of *Escherichia coli*: Rapid measurement by green fluorescent protein fluorimetry. J Bacteriol. **2007**;189(15):5601–7.

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