

Regulation of citrate utilisation in *Lactococcus lactis*

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Introduction

Citrate is an important precursor for flavour formation in dairy fermentations. It can be degraded into acetoin and diacetyl, which are responsible for the buttery flavour in dairy products. In *Lactococcus lactis* biovar diacetylactis citrate transport is facilitated by the plasmid-encoded citrate permease CitP. It is known that *citP* is induced in acidic environments. However, the role of citrate in the activation of citrate utilisation is still under debate and the plasmid copy number has never been taken into account.

The aim of this study was to decipher and quantify the role of different parameters in regulation of citrate utilisation (i.e. uptake and metabolism).

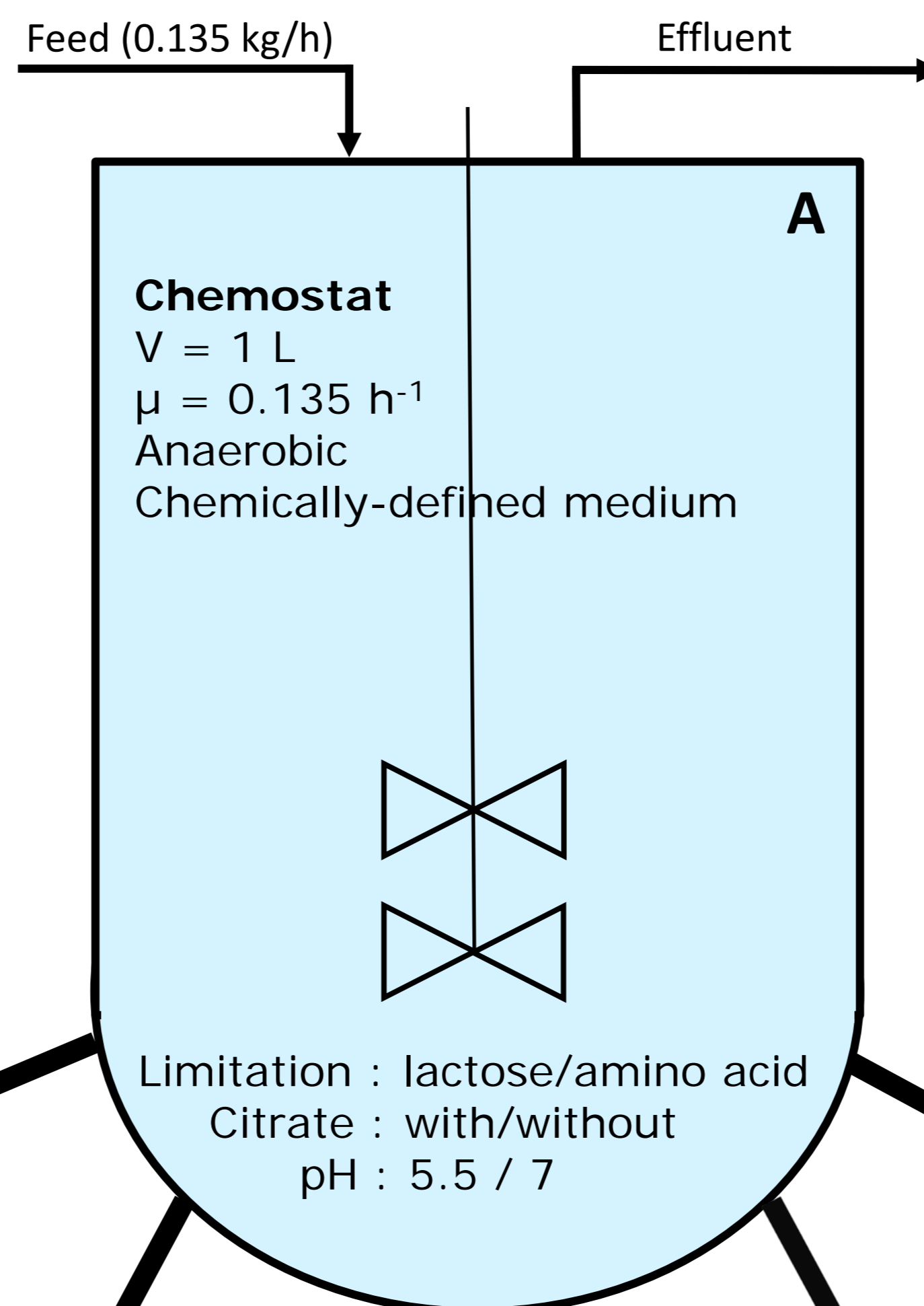
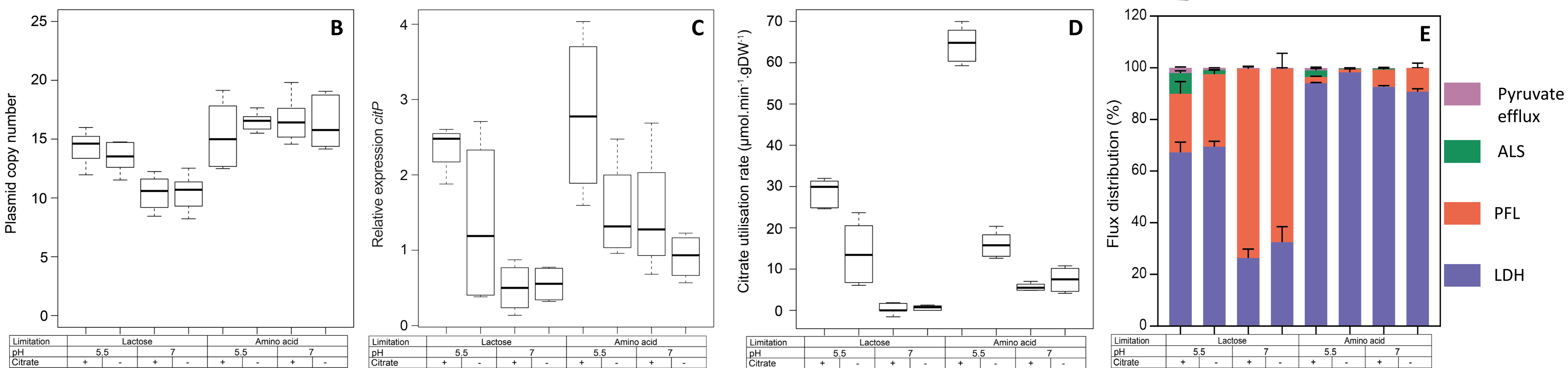


Figure 1.

A: Experimental design.
B: Plasmid copy number.
C: Relative expression of *citP*. Expression was corrected for the differences in plasmid copy number.
D: Citrate utilisation capacity.
E: Flux distribution of the main metabolic pathways with pyruvate as substrate.



- Plasmid copy number increased 1.5-fold under amino acid limitation and/or low pH
- Citrate did not significantly affect plasmid copy number

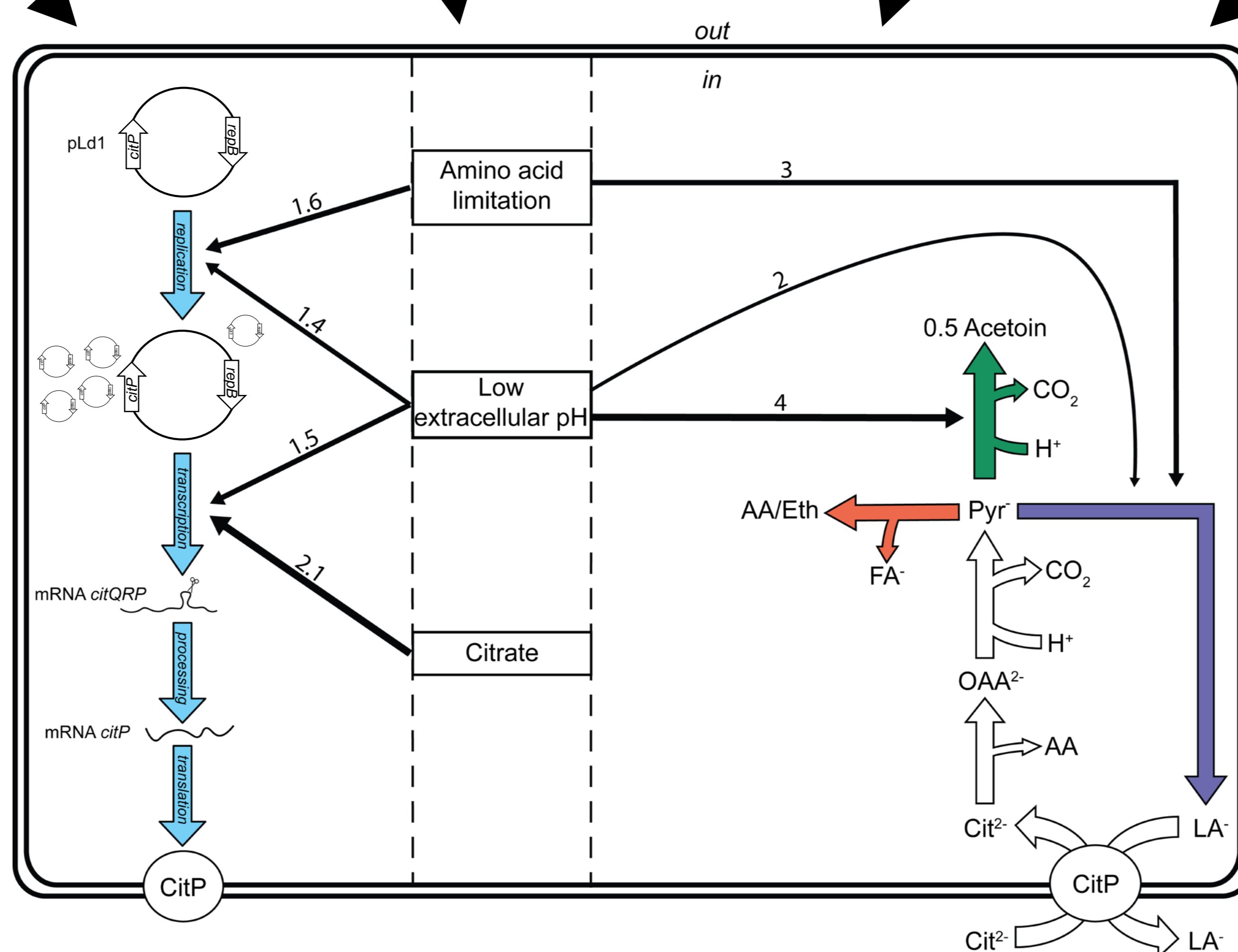
- citP* expression 5-fold higher at low pH with added citrate
- No effect amino acid limitation

- >100-fold increase in citrate utilisation capacity in cells with high *citP* expression

- Lactate major end-product under amino acid limitation
- Mixed acid fermentation under lactose limitation
- Acetoin mainly produced at low pH with added citrate

Figure 2.

Overview of the regulation of citrate uptake and metabolism in *Lactococcus lactis* biovar diacetylactis by nutrient limitation, pH and citrate. Black arrows represent a positive influence on the process or reaction. Numbers above arrows represent relative contributions. Abbreviations: Cit²⁻: divalent citrate; LA⁻: lactate; FA⁻: formate; OAA⁻: oxaloacetate; Pyr⁻: pyruvate; AA: Acetic acid; Eth: ethanol



Conclusions

Citrate utilisation in *Lactococcus lactis* is regulated by the presence of citrate, the pH and amino acid limitation. Amino acid limitation affects the plasmid copy number, while citrate and a low pH induce transcription of *citP*. The effect of citrate has been overlooked by other researchers because they used M17 medium which already contains 0.80 ± 0.07 mM citrate. The knowledge obtained in this study will help to select conditions to improve flavour formation from citrate.

Publication

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