Lactate morbidostat: A novel approach in ALE to improve lactate resistance of LAB

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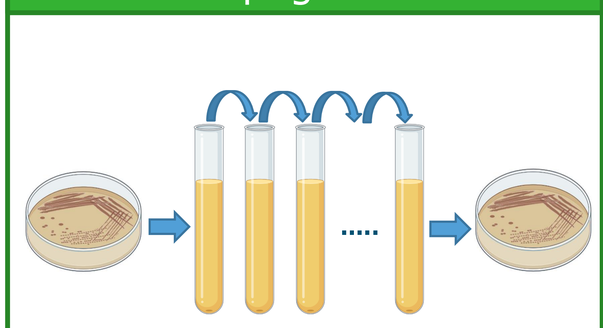
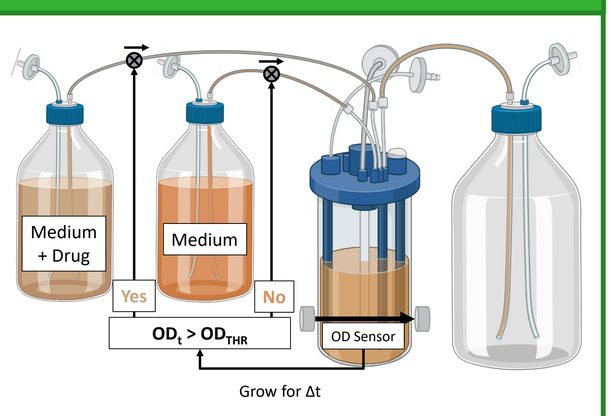
Background

End-product inhibition by lactic acid (LA) is the major limiting factor in the production of lactic acid bacteria (LAB) for starter cultures. Despite applying pH-control, lactic acid inhibits bacterial growth, resulting in decreased biomass productivity, titer and yield. Neither the mechanism of inhibition by lactic acid, nor the strategy of LAB to grow under lactic acid stress is well described. Adaptive laboratory evolution (ALE) provides a top-down approach to overcome this knowledge gap.

Introduction

ALE is a scientific approach to analyze evolutionary phenomena in controlled laboratory settings. In ALE, microorganisms are cultivated for prolonged periods under a selective and defined environment. This allows the selection of beneficial mutation(s) for that specific environment. In general, there are 2 main methods of ALE: (1) sequential batch propagations, and (2) continuous cultivation. One of the variations of with continuous cultivation is the morbidostat. Morbidostat has been developed by Toprak *et al.*[1] to study the evolution of drug resistance in microorganism. This system maintains constant evolutionary pressure despite the occurrence of mutation(s). Inspired by the morbidostat, we developed the lactate morbidostat as novel ALE method to increase lactic acid resistance of LAB.

Table 1. Overview of ALE methods: sequential batch propagations vs morbidostat

Sequential Batch Propagations	Continuous Culture: Morbidostat
	
Selection pressure	
• High growth rate • Survival in stationary phase	• Growth at high drug concentration
Bottleneck	
• Uncontrolled environment (decrease in pH) • Limited generations	• Biofilm formation • Laborious setup

Objectives

- To develop a novel ALE method, coined as lactate morbidostat
- To use this system to select LAB mutant(s) with improved lactic acid resistance

Innovation: Lactate Morbidostat

Lactate morbidostat expands the use of chemostat in ALE from selection for increased substrate affinity to selection for improved resistance towards end-product inhibition. In contrast to the classical chemostat, there is no substrate limitation in the lactate morbidostat. Instead, a constant inhibitory concentration of lactic acid is applied. During the fermentation, the lactic acid concentration increases *in situ* through LAB fermentation in contrast to the external input applied in a drug resistance morbidostat. The steady state is reached due to lactate inhibition and the increase in base addition rate indicates the occurrence of mutants with higher lactate resistance.

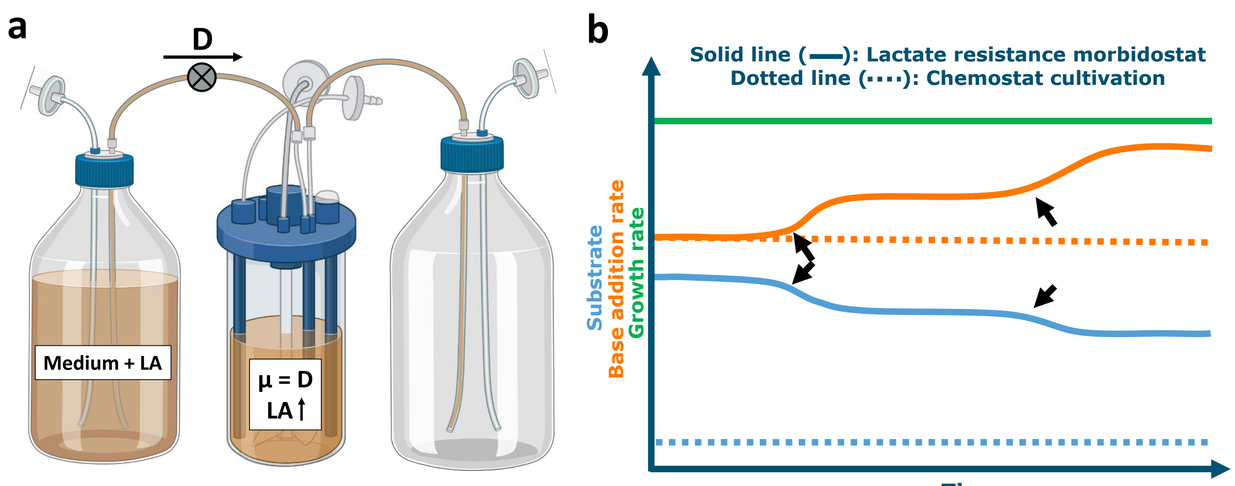


Figure 1. (a) Lactate morbidostat setup. This system requires only one medium reservoir containing a surplus of substrate and the inhibitory compound, lactic acid. The dilution rate remains constant and determines the growth rate. **(b)** Expected outcome of lactate morbidostat. Substrate consumption increases every time a mutation that improves the microorganism fitness occurs (black arrows). This will lead to more LA produced and thus increase the stress condition in the culture. The occurrence of this adaptive process is monitored online by measuring base addition rate over time.

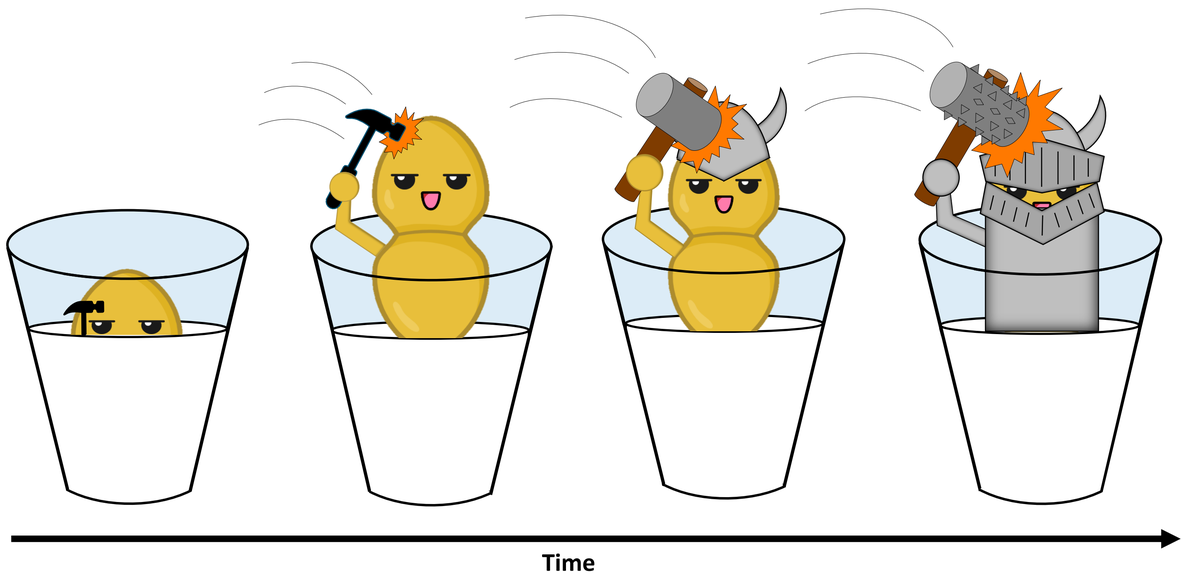


Figure 2. Lactate morbidostat illustrated as self-whack-LAB game. LAB culture is grown in the continuous cultivation. LAB produce lactic acid during growth that causes growth inhibition (shown as hammer). Prolonged cultivation in high lactic acid generates mutation(s) that increases LAB resistance (shown as armor). Multiple mutations (better armor) provide higher resistance and improved LAB growth, under conditions where more lactic acid is produced causing increased stress conditions (bigger hammer). *Inspired by Fridman et al. illustration of morbidostat as a whack-a-mole game* [2].

Acknowledgements

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References

1. Toprak, E., et al., *Evolutionary paths to antibiotic resistance under dynamically sustained drug selection.* Nat Genet, 2012. **44**(1): p. 101-5.
2. Fridman, O., A. Goldberg, and N.Q. Balaban, *Whack-an-E. coli with the morbidostat.* Genome Biol, 2012. **13**(1): p. 140.

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