Micro-droplet based directed evolution outperforms conventional laboratory evolution

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MICRO-DROPLET BASED DIRECTED EVOLUTION OUTPERFORMS CONVENTIONAL LABORATORY EVOLUTION

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ABSTRACT

We present droplet adaptive laboratory evolution (DrALE), a directed evolution method used to improve industrial enzyme producing microorganisms for e.g. feedstock digestion. DrALE is based linking a desired phenotype to growth rate allowing only desired cells to proliferate. Single cells are confined in microfluidic droplets to prevent the phenotype, e.g. secreted enzymes, from leaking between cells.

The method was benchmarked against and found to significantly outperform conventional adaptive laboratory evolution (ALE) in enriching enzyme producing cells. It was furthermore applied to enrich a whole-genome mutated library of yeast cells for α-amylase activity.

KEYWORDS: Directed evolution, Droplet microfluidics, High throughput, Enzymes, Cell factories

INTRODUCTION

Enzyme producing microorganisms are important to a wide range of industrial applications. As an alternative to classic chemical catalysis they have the advantages of being more environmentally friendly. Directed evolution towards improved enzymatic microorganisms can be accomplished with conventional means such as ALE[1] or with miniaturized droplet microfluidic sorting systems[2]. Recently, it was demonstrated that by growing cells in droplets, selection could be achieved for slow but efficient strains as resources are evenly aliquoted to each cell[3]. Here we introduce DrALE, a novel method for directed evolution of enzyme producing microorganisms based on clonal expansion in micro-droplets whereby only cells with desired enzymatic activity can access nutrients and grow.

THEORY

DrALE relies on encapsulation of single cells into microfluidic droplets containing a defined media (Figure 1). The sole carbon source in the media is supplied by the complex carbohydrate, e.g. starch, that the cells are evolved towards being able to digest. In DrALE, only cells that can efficiently digest the complex carbohydrate to release sugars can proliferate (Figure 2). In contrast, in ALE (i.e. flask culture), the released sugars become a public good that any cell can consume (Figure 3). After the cells have expanded inside the droplets, the emulsion is broken and the process can be iterated for several rounds to enrich for even higher enzymatic activity.

EXPERIMENTAL

Yeast cells were suspended in defined media with starch as sole carbon source. Cell suspension was diluted to OD_{600}=0.2 and single yeast cells were encapsulated into monodisperse 320pL sized droplets in HFE7500 oil with 1%(w/v) EA surfactant on a microfluidic chip at 440Hz. Droplets were incubated at 30°C to allow for cell growth. Emulsion was broken by adding 10%(v/v) 1*H*,1*H*,2*H*,2*H*-Perfluoro-1-octanol, the water phase (containing the cells) was diluted washed and then diluted in fresh starch media for further rounds of DrALE. Samples were frozen from each round of evolution for analysis. α-amylase activity was measured by cultivating samples in glucose media, washing the cells and inoculating the samples at a defined OD in micro titer plates with BODIPY-starch (Invitrogen).

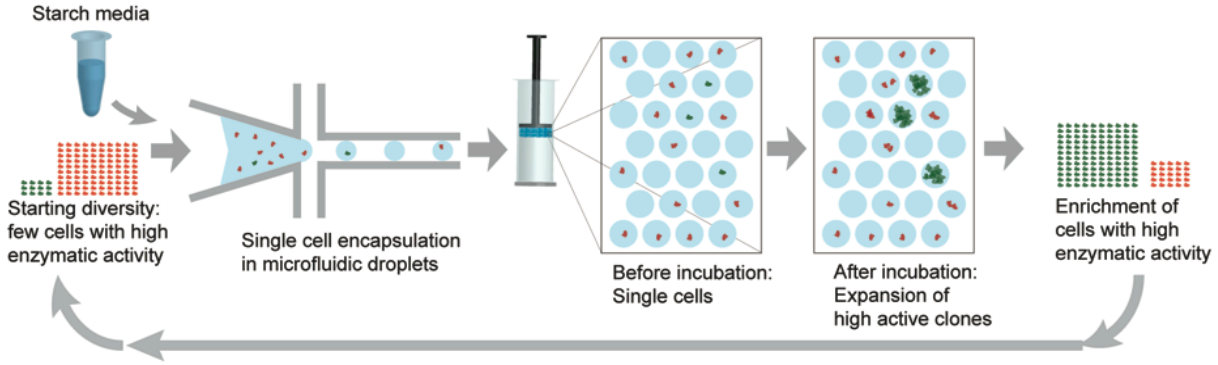

Figure 1: *Workflow schematic for droplet adaptive laboratory evolution (DrALE). A diverse cell population with various degrees of α-amylase production, i.e. a library is suspended in defined media with starch as sole carbon source. Cells are encapsulated into microfluidic droplets on a microfluidic device. Emulsion is collected and incubated in a syringe to allow for clonal expansion. Cell with high enzymatic activity liberate more sugar to support more growth. The workflow is iterated through several rounds to yield high producing strains.*

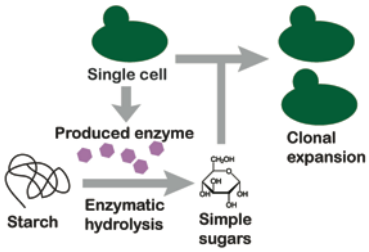

Figure 2: *Cells produce enzyme that can digest a complex carbohydrate to simple sugars. The simple sugars in turn provide energy for the cell to divide creating a feedback between enzyme production and cell growth.*

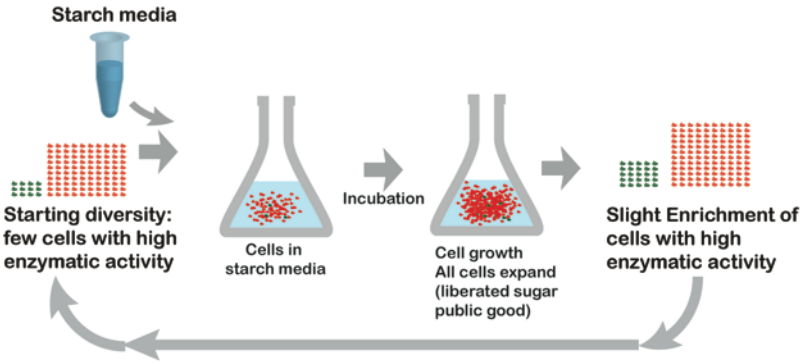

Figure 3: *Adaptive Laboratory Evolution (ALE). A diverse cell population is suspended in defined media with starch as sole carbon source. Cells with high enzymatic activity liberate more sugar to support more growth. However, since the sugar can freely diffuse around in the flask it may be consumed by other cells. This lowers the efficiency of the method and only slight enrichment of cells with high enzymatic activity was achieved.*

RESULTS AND DISCUSSION

The method was tested by mixing 2% α-amylase producing cells with 98% non-producers using one round of DrALE and ALE to enrich for α-amylase producers. α-amylase activity was measured to be 47% of the activity of α-amylase producing strain whereas the ALE culture had only 8%, indicating that DrALE provided better enrichment (Figure 4).

Furthermore, a whole-genome mutated cell library of α-amylase producing variants was enriched in parallel by both ALE and DrALE for four rounds. The α-amylase activity of the cultures were assayed from each round of evolution and compared to the original library as well as enhanced amylase producing strain B184 (Figure 5). The DrALE culture was found to have better enrichment than the ALE culture.

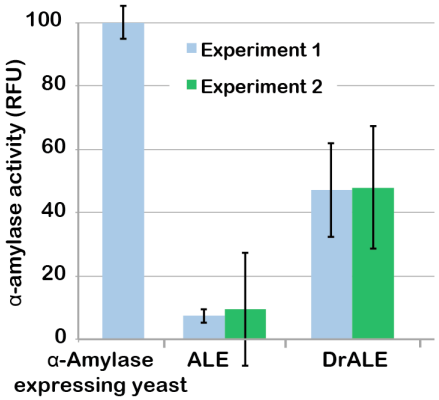

Figure 4: *α-amylase activity (cell count normalized) following one round of DrALE and ALE of a 2% α-amylase producer and 98% non-producer cell mixture. Experiment 1 and 2 are biological replicates.*

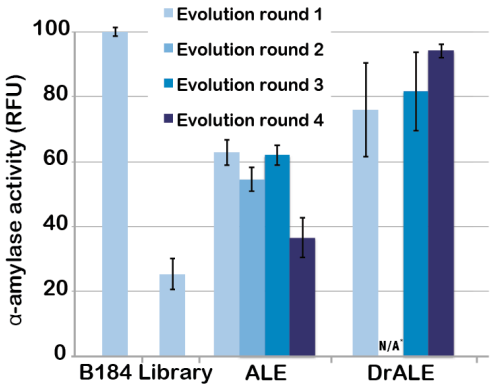

Figure 5*: α-amylase activity (cell count normalized) after 1-4 rounds of enrichment from a whole-genome mutated cell library using DrALE and ALE respectively. *The 2nd round activity measurement for the DrALE was lost because of technical error during readout.*

CONCLUSION

In summary, we demonstrate a novel method for directed evolution of microorganisms for feedstock digestion. In contrast to previous droplet-based directed evolution methods, our method provides a significantly higher throughput and does not rely on active droplet manipulation. Also, DrALE does not require a fluorescent substrate, greatly expanding the number of potential enzyme activities that can be addressed. In addition, the low fluidic complexity of the method makes it suitable for automation.

ACKNOWLEDGEMENTS

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