Investigating the Evolutionary Changes in Crabtree-negative Yeasts During a Long-term Evolution Experiment

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0.1 Abstract

The Crabtree effect is a metabolic strategy that allows yeast to ferment in the presence of oxygen. This is of interest as not all yeasts display this strategy, and nearly 100 years after its discovery it is still unclear what the overall benefit is. Two key theories attempt to explain the emergence of this phenomenon, the make-accumulate-consume theory and the rate/yield trade-off theory. The aim of this thesis was to investigate whether a trade-off between rate and yield develops in Crabtree-negative yeasts over the course of 1500 generations in a high sugar environment. Chapter Two demonstrates that growth rate is more likely to increase than decrease while growth yield is more likely to decrease than increase in the isolate-derived populations of yeast. We find that species that started out relatively fast, changed little while the slower species had more significant gains in growth rate. With growth yield, the species with initially high yield lost more significantly than the already low yield species. This could suggest there is an overall optimum growth rate and growth yield, that the species are evolving towards. In Chapter Three, ethanol production was measured using colorimetric tests and no change was observed to support the development of the Crabtree effect in these populations after 1500 generations. In Chapter Four growth yield was investigated using flow cytometry and it was found that several yeast populations both increased in cell size and decreased in growth yield. This is an interesting observation that has been observed in several previous experimental evolution experiments. In Chapter Five, as cell size is often associated with ploidy changes, DNA content was measured using DAPI and SYTOX DNA stains, detected by flow cytometry. This did not provide any statistically significant conclusions but highlighted the importance of employing further techniques to analyse the DNA content of these populations. This thesis has illustrated the importance of studying the competitive behaviours of microorganisms in isolation, where selfish traits appear to thrive.

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0.3 Table of Contents

0.4 Abbreviations

ADH	Alcoholic dehydrogenase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CFU	Colony-forming units
DAPI	4',6-diamidino-2-phenylindole
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ESS	Evolutionary stable strategy
FACS	Fluorescence activated cell sorter
FSC	Forward-scattered light
GTP	Guanosine-5'-triphosphate
HPLC	High-performance liquid chromatography
HXT	Hexose transporter
MAC	Make-accumulate-consume
NADH	Nicotinamide adenine dinucleotide
OD	Optical density
PBS	Phosphate-buffered saline
RPM	revolutions per minute
RYT	rate/yield trade-off
SM	synthetic minimal

TCA	Tricarboxylic acid
WGD	Whole genome duplication
YPA	Yeast peptone agar
YPD	Yeast extract peptone dextrose

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