

## Supplementary figure 2.3

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  "PRJNA102289": {
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    "title": "Prolonged selection in aerobic, glucose-limited chemostat cultures of Saccharomyces cerevisiae",
    "description": "Prolonged cultivation of Saccharomyces cerevisiae in aerobic, glucose-limited chemostat cultures (dilution rate, 0.10 h-1) resulted in a progressive decrease of the residual glucose concentration (from 20 to 8 mg l-1 after 200 generations). This increase in the affinity for glucose was accompanied by a fivefold decrease of fermentative capacity, and changes in cellular morphology. These phenotypic changes were retained when single-cell isolates from prolonged cultures were used to inoculate fresh chemostat cultures, indicating that genetic changes were involved. Kinetic analysis of glucose transport in an 'evolved' strain revealed a decreased Km, while Vmax was slightly increased relative to the parental strain. Apparently, fermentative capacity in the evolved strain was not controlled by glucose uptake. Instead, enzyme assays in cell extracts of the evolved strain revealed strongly decreased capacities of enzymes in the lower part of glycolysis. This decrease was corroborated by genome-wide transcriptome analysis using DNA microarrays. In aerobic batch cultures on 20 g glucose l-1, the specific growth rate of the evolved strain was lower than that of the parental strain (0.28 and 0.37 h-1, respectively). Instead of the characteristic instantaneous production of ethanol that is observed when aerobic, glucose-limited cultures of wild-type S. cerevisiae are exposed to excess glucose, the evolved strain exhibited a delay of 90 min before aerobic ethanol formation set in. This study demonstrates that the effects of selection in glucose-limited chemostat cultures extend beyond glucose-transport kinetics. Although extensive physiological analysis offered insight into the underlying cellular processes, the evolutionary 'driving force' for several of the observed changes remains to be elucidated. Keywords: evolution Overall design: A crucial feature of bakers' yeast is its capacity to produce CO2, referred to as fermentative capacity (van Hoek et al., 1998). After prolonged glucose-limited cultivation of S. cerevisiae, in addition to an increased affinity for glucose, we observed a dramatic decrease in fermentative capacity. Consequently, the aim of the present study was to perform an integral analysis of the long-term adaptation of S. cerevisiae during prolonged glucose-limited, aerobic cultivation in chemostat cultures, with special emphasis on the regulation of glucose transport and glycolytic capacity. To this end, we applied an integrated approach that combined transcriptome analysis, measurement of fermentative capacity and activities of glucose transport and glycolytic enzymes, and characterization of cellular morphology.",
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