Strain and Culture Conditions

The haploid yeast *Saccharomyces cerevisiae* CEN.PK113-7D, obtained from the *Centraalbureau van Schimmelcultures* (Fungal Biodiversity Center, Utrecht, The Netherlands), was used in this study. The cultivation was performed using a low-salt Verduyn minimal medium [1] with a glucose concentration of 7.5 g/L. 1L-Erlenmeyer flasks containing 100 mL medium were inoculated with cells from a cryovial (glycerol, -80oC) and the inoculation cultures were subsequently grown for 10 h at 200 rpm and 30 °C. The inoculation culture was used to inoculate a 7 L bioreactor (Applikon, Schiedam, The Netherlands) containing a working volume of 4 L. The reactor was aerated with pressurized air at 1 L/min (0.25 vvm) using a Smart series mass flow controller 5850S (Brooks Instrument, PA, USA). The reactor was operated at 0.3 bar overpressure, at 30oC, with a stirrer speed of 600 rpm. The pH of the broth was maintained at 5.0 by adding either 4M KOH or 2M H2SO4. Once the batch phase was completed (indicated by a fast decrease in CO2 signal and a sharp increase in dissolved oxygen (DO)), the chemostat phase (steady-state) was started at a dilution rate of 0.1 h-1 for 50 h. After about 5 residence times, sampling for proteomics was performed.

Dynamic Feast Famine Setup

After five residence times (50 h) of chemostat feeding, the regime was changed to a so-called repetitive feast/famine phase [2], during which Successive cycles of 400 s were applied by a continuous medium feeding for 20 s, followed by a period of 380 s of no feeding. The medium pump was controlled using an automatic timer (PTC-1A, Programmable timing controller, Omega Engineering Inc., Stamford, CT, USA). During the 20-second feeding period, 43 ± 1 mL of fresh medium were added. The same volume was subsequently withdrawn during the first 260s at a flow rate of 0.166 ± 0.001 mL s−1 maintaining the broth volume nearly constant at 4 L. After about 5 residence times, sampling for proteomics was performed.

Proteomics analysis

For each sample, a sample normalized to 10 OD units was withdrawn into an eppendorf tube and immediately centrifuged (Heraeus Biofuge Stratos centrifuge) at 8000 g for 5 min at 4oC. Supernatant was discarded and the pellet was subsequently resuspended in 2 mL saline solution (0.9% NaCl) (cooled beforehand at 4oC) and centrifuged again (8000 g, 5 min, 4oC). The supernatant was discarded once more, again resuspended in 2 mL saline solution and centrifuged (8000 g, 5 min, 4oC). After the third centrifugation, the supernatant was discarded and the sample was snap-frozen using liquid nitrogen and stored at -80oC until further analysis. For each timepoint, duplicate samples were taken.

Proteomics measurements were performed and data were subsequently mapped using the proteome database from *Saccharomyces cerevisiae* (downloaded from Uniprot, *S.cerevisiae* CEN.PK113-7D, ID:UP000013192, December 2019) [3] by DSM, Delft, The Netherlands. The data was analysed for statistical differences using Perseus 1.6.10.45 [4]. A two sample test was used to determine the significance of the fold change, with a significance level threshold of p < 0.01, and at least 2 unique peptides per protein.

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