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Genomic analysis of *Saccharomyces cerevisiae* isolates that grow optimally with glucose as the sole carbon source

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Abstract

A population of *Saccharomyces cerevisiae* was cultured for approximately 450 generations in the presence of high glucose to select for genetic variants that grew optimally under these conditions. Using the parental strain BY4741 as the starting population, an evolved culture was obtained after aerobic growth in a high glucose medium for approximately 450 generations. After the evolution period three single colony isolates were selected for analysis. Next-generation Ion Torrent sequencing was used to evaluate genetic changes. Greater than 100 deletion/insertion changes were found with approximately half of these effecting genes. Additionally, over 180 single-nucleotide polymorphisms (SNPs) were identified with more than one quarter of these resulting in a non-synonymous mutation. Affymetrix DNA microarrays and RNseq analysis were used to determine differences in gene expression in the evolved strains compared to the parental strain. It was established that approximately 900 genes demonstrated significantly altered expression in the evolved strains relative to the parental strain. Many of these genes showed similar alterations in their expression in all three evolved strains. Interestingly, genes with altered expression in the three evolved strains included genes with a role in oxidative metabolism. Overall these results are consistent with the physiological observations of optimal growth with glucose as the carbon source. Namely, the decreased ethanol production suggest, that the underlying metabolism switched from fermentation to respiration during the selection for optimal growth on glucose.

Introduction

Most living cells metabolize organic compounds, such as carbohydrates, to generate the metabolic precursors to support growth. During metabolism, the most efficient way to generate chemical energy (adenosine triphosphate - ATP) is thorough aerobic respiration. In their natural environment yeast can utilize a wide variety of carbon sources to support cellular growth and survival; however, they prefer to metabolize hexose sugars such as fructose or glucose. The yeast *Saccharomyces cerevisiae* is unique in the fact that during aerobic growth, in the presence of high glucose, it utilizes fermentation (lower ATP production) rather than respiration (higher ATP production. This phenomenon is referred to as the Crabtree effect where yeast produces ethanol through fermentation when grown aerobically in the presence of high levels of glucose [1]. In yeast, this phenomenon is thought to have evolved to increase the fitness and survival by minimizing cellular expenditures [2].

Aerobic respiration is the set of metabolic reactions that cells utilize to oxidize organic compounds into ATP and CO_2. The citric acid cycle is a key component of these metabolic pathways by which all organisms generate redox potential that is used to generate ATP. The vast majority of organisms utilize this cycle to break down glucose to produce energy when growing aerobically and only utilize fermentation during anaerobic conditions. For example,

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fermentation occurs in mammalian muscles during periods of intense exercise where oxygen supply becomes limited, resulting in the formation of lactic acid [3]. Conversely, *S. cerevisiae* only reverts to respiration when glucose becomes limiting [1].

Because yeast has the ability to adapt to a variety of growth conditions we tested the hypothesis that if yeast are cultured for an extended period of time in the presence of high glucose they will adapt to utilize respiration rather than fermentation during aerobic growth. Using genome scale techniques we determined the effects of continued growth of yeast in the presence of high glucose concentrations. First, using next generation sequencing we examine the genome sequence variations that occurred relative to the un-evolved parental strains. Second, we used RNAseq and microarray analysis to identify the mRNAs whose abundance differed between the parental and evolved strains. Finally, through this analysis we present evidence that supports our hypothesis that when exposed to high levels of glucose for a prolonged period of time yeast will adapt to using reparation rather than fermentation.

Materials and Methods

Cell growth

Cultures of parental strain BY4742 were grown in 100 ml of CM Broth with Glucose (2% glucose, 0.5% Ammonium Sulfate) at 30°C with aeration. Every day for a month 10μl of the culture was transferred to a flask with fresh medium to maintain the culture in maximal exponential growth phase. Using Precision Laboratories Glucose Test Strips (Fisher Scientific) the remaining culture was tested for the level of glucose present to ensure that the cells had not completely exhausted the carbon source and therefore the concentration remained relativity high. Additionally, the amount of ethanol produced was measured daily using Ethanol Assay, UV-method kit (R-Biopharm). Finally, the cells were observed under a microscope to ensure that there was no contamination. After the 30 day evolution period, a portion of the culture was grown on YPD (1% yeast extract, 2% peptone, 2% D-glucose) plates. After overnight growth three single colonies were selected. Theses colonies were then cultured over night in CM Broth with Glucose. To collect cells, 50 ml of culture was placed into a 50 ml conical tube, and centrifuged for 5 minutes at 3,000 rpm. After centrifugation the culture was washed with sterile water and aliquoted into 1.5 ml tubes, centrifuged for 3 minutes at 8,000 rpm, the water was aspirated off and the pellets were stored at −80°C to be used for DNA or RNA isolation.

DNA Isolation

DNA was isolated from all strains using QIAamp® DNA Mini kit (Qiagen) as per manufacture instructions. The frozen pellet was resuspended in 180 μl ATL reagent and 20 μl proteinase K was added. The sample was vortexed and incubated at 56°C for 4 hours, with inversion of the tube every 30 minutes. After incubation the sample was pulse spun and 4 μl RNase A (100 mg/ml) was added, samples were vortexed and pulse spun then incubated at RT for 2 minutes. After incubation, 200 μl of Buffer AL was added, vortexed, pulse spun and then incubated at 70°C for 10 minutes. Following incubation, 200 μl of 100% ethanol (96–100%) was added and mixed by pipetting up and down. The entire mixture was then placed in the QIAamp Mini spin column and centrifuged at 10,000 rpm for 1 minute. The flow through was discarded and 500 μl Buffer AW1 was added to the spin column then centrifuged for 1 minute at 10,000 rpm. The flow through was discarded and 500 μl Buffer AW2 was added and centrifuged at 13,000 rpm for 1 minute. The flow through was discarded and the mini spin column was centrifuged dry for 2 minutes to eliminate all wash buffers before elution step. The mini-column was placed in a clean 1.5 ml non-stick tube and 200 μl Buffer AE was added to the center of the column. The column was incubated for 2

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minutes at room temperature then spun at 13,000 rpm for 1 minute to elute DNA. DNA concentration was determined using the nanodrop.

RNA isolation

Total RNA was isolated using RiboPure™ yeast kit (Life Technologies) as per manufacture instructions. Briefly, frozen pellets were thawed and resuspended in 480 µl Lysis Buffer, 48 µl 10% SDS, and 480 µl of Phenol:Chloroform:Isoamyl Alcohol (Sigma-Aldrich). The mixture was vortexed and added to 750 µl cold Zirconia Beads. Cells were placed on a Labnet Orbital Shaker at top speed for 10 minutes. Cell debris was removed by centrifugation at 14,000 rpm for 5 minutes at room temperature. The aqueous phase was transferred to a new 5 ml tube. The pellet was discarded. 1.9 mls of Binding Buffer was added to the 5 ml tube with aqueous and mixed thoroughly by vortexing. 1.25 mls of 100% ethanol was added and mixed by vortexing. 700 µl of the mixture was loaded onto a filter cartridge and centrifuged at 13,000 rpm for 30 seconds. Flow through was discarded and this process was continued until the entire amount of sample was loaded into the cartridge. The filter was then washed once with 700 µl of Wash Solution 1, centrifuged at 13,000 rpm and flow through was discarded. The filter was washed twice with 500µl Wash Solution 2/3 with centrifugation at 13,000 rpm for 30 seconds and flow through discarded between washes. The column was spun dry for 1 minute at 13,000 rpm. The filter cartridge was placed in a new Nuclease free 1.5 ml tube. 50 µl of pre-heated Elution Solution was added to the center of the filter and centrifuged for 1 minute at 13,000 rpm. An additional 50 µl of pre-heated elution solution was added to the center of the filter and spun at 13,000 rpm for 1 minute. The 100 µl of RNA was then treated with 4µl of DNase I for 30 minutes at 37°C. The reaction was stopped using 11 µl DNase Inactivation Reagent and incubated at RT for 5 minutes. Sample was then centrifuged at 13000 rpm and the RNA was removed from the DNA inactivation pellet and placed in a new Nuclease free 1.5 ml tube. RNA was examined for degradation with Agilent 2100 Bioanalyzer instrument using the RNA 6000 Pico Kit. Isolated RNA was stored at −80°C and subsequently used for microarray and RNAseq experiments.

Afflymetrix Microarray and Analysis

For array analysis the DNA from each strain was labeled using the Affymetrix GeneChip DNA Labeling Reagent as per manufacture protocol. After labeling each sample was hybridized to a GeneChip Yeast Genome 2.0 Array for 17 h at 45°C with constant rotation. The chips were then washed and scanned using GeneChip® Scanner 3000. Two biological replicates were carried out for each strain. The data was then exported to GeneSpring v 11.5.1 and normalized using a set of 100 'housekeeping genes', which are re-scaled so the average values of these genes are equal across all the microarrays used in the experiment. This normalization is carried out to adjust for variations that may be introduced due to the technology rather than the biological condition being examined. Using the normalized data, the mRNAs that differed in abundance between the parental strain and evolved strains were then identified using GeneSpring v 11.5.1 using two steps. First, all genes that have a value of 100 were considered in the "noise" and were removed from any further analysis. Second, the remaining genes were then analyzed to determine those that differed by at least two fold between the parental and each of the evolved strains. Those genes were then considered to be differentially regulated between the strains. To determine the reproducibility between biological replicates the log2 values were exported from GeneSpring v 11.5.1 and plotted using Excel where an R^2 values were generated. The R^2 values were greater than 0.98 for all of the strains, indicating that there is minimal variability across the biological replicates. All array data has been uploaded to the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE36518.

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Whole Genome Library Preparation and Analysis

To carry out whole genome resequencing a library was produced from isolated DNA using the Ion Xpress™ Fragment Library Kit (Life Technologies) per manufacture protocol. Briefly, DNA was fragmented using the Ion Shear™ Reagents incubating at 37°C for 30 minutes. The fragmented DNA was then purified using the Agencourt® AMPure® XP Kit (Beckman Coulter). The fragmented DNA was eluted with 50 μl RNase free water. After purification, adaptors were ligated to the fragmented DNA. The ligation product was purified using the Agencourt® AMPure® XP Kit. The purified DNA was then run on a 6% polyacrylamide gel; the ligation was then size selected and excised from the gel and eluted in PAGE elution buffer at 50°C overnight. The library was then purified by completing a phenol:chloroform:isoamyl alcohol purification and 100% ethanol precipitation and resuspended in 50 μl low TE. The purified and size selected DNA was then PCR amplified. The final library was purified using the Agencourt® AMPure®XP Reagent. Final library DNA was eluted in 20 μl Low TE and stored at −20°C. DNA concentration was determined using the Qubit® 2.0 Fluorometer. Whole genome resequencing data was analyzed using CLC Genomic Workbench 4.8. sequence reads were mapped back to the s288c genome reference sequence (NC_001133 - NC_001148 and NC_12224). To determine if each called SNPs and DIPs were "real" they were required to have at least 4× coverage and be present in at least 100% of the reads covering that area. Additionally, the SNPs and DIPs were further verified by examining the surrounding sequence to ensure that the call was not in a region that contained a homopolymer repeat before or after the base. This is because Ion Torrent sequencing can have miscalls in homopolymeric regions. Sequence data has been uploaded to the Sequence Read Archive (http://trace.ncbi.nlm.nih.gov/Traces/sra/) under accession number SRA050713.

mRNA Library Preparation and Analysis

To carry out RNAseq analysis, a library was produced from isolated total RNA. The Ion Total RNA-Seq Kit (Life Technologies) was used to produce mRNA libraries. Using this kit 500 ng of total RNA was used to construct the libraries. Briefly, total RNA was fragmented using RNase III by incubating the reaction at 37°C for 10 minutes. The fragmented RNA was then purified using the RiboMinus™ Concentration Module (Invitrogen). The yield was then determined using the Qubit® RNA Assay Kit (Invitrogen) with the Qubit® Fluorometer. The adaptor sequences were then hybridized and ligated to the mRNA. A reverse transcription reaction was carried out to produce cDNA. The synthesized cDNA was purified and size selected using 2 rounds of purification with the Agencourt AMPure® XP reagent kit (Beckman Coulter). The purified cDNA was then amplified with 16 cycles of PCR. The amplified cDNA was purified using the PureLink® PCR Micro Kit (Invitrogen) and quantified using Qubit® DNA Assay Kit to determine the DNA concentration and subsequent dilution factor for the library. RNAseq data was analyzed using CLC Genomic Workbench 4.8. To determine the genes that were differentially regulated a Gene Set Enrichment Analysis (GSEA) was carried out. Using a standard t-test GSEA determined the association between the genes and then ranked the genes based on their association. These rankings were then used to determine whether members of a gene set were toward the top or bottom of the list for each of the strains.

Emulsion PCR and Next Generation Sequencing

Whole genome and mRNA Sequencing was carried out using an Ion Torrent Personal Genome Machine. Using the Ion Torrent sequencing system we were able to sequence the four strains (Parental BY4741 and evolved strains ES1, ES2, and ES3). The genomic and mRNA libraries were clonally amplified on to IonSphere particles for sequencing. Briefly, the diluted library was added to washed Ion Sphere™ Particles and the PCR master mix. All components were added to the oil phase and mixed using an IKA® Ultra-Turex® Drive to

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generate the emulsion. The emulsion was then placed in a 96 well plate and PCR was carried out for 45 cycles. The Ion Sphere™ particles were recovered by placing the emulsion in a breaking solution consisting of 9 ml of Recovery Solution into 3 ml of 1-butanol. The samples were washed with recovery solution and then enriched to remove any Ion Sphere™ without DNA. All clonally amplified samples were then sequenced on the Ion Torrent Personal Genome Machine using 314 or 316 chips.

Results

Ethanol production over time

To investigate the ability of yeast cultured in a high glucose environment to evolve toward optimal growth, we monitored the switch from fermentative metabolism to respiration by examining the level of ethanol produced (Figure 1). Using a UV based measurement approach, it was established that the amount of ethanol produce varied from 6-8 g/l during exponential growth during the first 16 days. Interestingly, between days 17 and 21 there was a dramatic decrease in the amount of ethanol produced. Beyond day 22 there was no detectable ethanol being produced by the yeast cells. These results support the hypothesis that over time yeast cells growing in a glucose rich environment can evolve to adopt respiration over fermentation.

DNA sequencing of an evolved yeast strain

To determine genetic changes in the evolved strains relative to the parental strain we performed whole genome resequencing using the Ion Torrent PGM [4]. Greater than 3 million single-end, ~100 base pair reads were generated for each strain. Using CLC Genomic Workbench 4.8, it was determined that more than 80% of the final reads were aligned to the *S. cerevisiae* (S288c) reference genome [5] for each specific yeast strain. After quality-filtering was carried out, these reads yielded a coverage of >99.9% of the mappable nuclear and mitochondrial genome of the laboratory-evolved strains at >24× depth coverage.

To further investigate the genetics underlying the evolved strains a detailed analysis of the deletion/insertion polymorphisms (DIPs) as well as the single-nucleotide polymorphisms (SNPs) was completed. DIPs were identified as being common across the three evolved strains (Table 1). Although there were no genes mutated that could explain this phenotype there were still several interesting mutations in this group. These include *SCW11,* a previously characterized cell wall protein believed to play a role in mating [6]. In addition *YCF1* a vacuolar glutathione S-conjugate transporter that is involved in detoxifying heavy metals [7]. Finally, there were seven proteins of unknown function found, which included the putative transporter YDR387c.

Specific DIPs were not identified that could potentially explain the phenotype of the evolved strain. Therefore further analysis of the SNPs identified was completed (Table 2). From these 27 non-synonymous SNPs were determined across three isolates of the evolved strains. Of these, 3 previously uncharacterized genes were identified; however, previously characterized genes with interesting functions were identified. These include *HXT2*, a glucose transporter [8] that is known to be repressed when normal yeast cells are growing in a high glucose environment. Additionally, *VRG4,* a Golgi GDP-mannose transporter [9] and *MDS3*, a putative component of the TOR regulatory pathway [10] were identified. Interestingly, the TOR pathway is known to be involved in the regulation of growth as well as nutrient sensing in yeast [11]. Furthermore, YPL260w, which is a putative substrate of cAMP-dependent protein kinase (PKA) [12] was also identified, where PKA is known to regulate cellular growth in response to available nutrients [13]. Finally, a mutation in *PKC1*,

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which regulates cell growth [14] was established. Collectively, these results suggest that the observed phenotype is potentially due to the overall effect of the specific mutations on cellular growth and survival.

RNAseq analysis of an evolved yeast strain

To further examine the differences between the evolved strain and its parental strain, we used the Ion Torrent sequencer to carry out RNAseq analysis to identify the genes whose mRNA abundance was significantly altered between the strains. We analyzed three biological replicates of each strain using RNAseq. Using CLC Bio Gene Enrichment Analysis (GSEA), a number of significant differences between the strains were determined (Table 3). The genes differentially expressed fell into several interesting categories, include genes involved in the tricarboxylic acid cycle (TCA), aerobic respiration, as well as signal transduction. These results further support the hypothesis that the evolved strain had preference for respiration rather than fermentation when grown in a high glucose environment.

Microarray analysis of evolved yeast strains

To further expand on and verify the RNAseq data, Affemetirx microarrays were used to determine what specific genes were differentially expressed across all three evolved strains relative to the parental strain BY4741. To determine the overlap between the strains, we compared transcripts from each evolved strain relative to the parental. From the microarray analysis we found over 900 mRNAs that are differentially regulated between the evolved strain and its parental (Fig 2). Analyzing two biological replicates of each strain using the microarrays; in total 985 mRNAs were identified in common across all three evolved strain isolates, with 476 mRNAs increasing in abundance (Fig 2A) and 509 decreasing in abundance (Fig 2B) in the evolved strain relative to its parental. Further examination of these genes, using GO term finder at *Saccharomyces* genome database (SGD) [15], revealed significant number of genes in a variety of metabolic categories. These categories included carboxylic acid metabolic process, cellular amino acid metabolic process and small molecule metabolic and biosynthetic process.

Although there were no similar categories between the RNAseq and microarray analysis in terms of significance a closer look at the genes revealed some interesting overlaps. There were three genes identified that encode proteins that have roles in the TCA cycle. These included the Pyruvate kinase *CDC19* that converts phosphoenolpyruvate to pyruvate during glycolosis [16]. The mitochondrial aconitase isozyme, *ACO2*, which is required for the TCA cycle in yeast [17]. Finally, the Alpha subunit of succinyl-CoA ligase, *LSC1* that functions in the conversion of succinyl-CoA to succinate [18]. Additionally through the microarray analysis several genes that encode proteins that function in cellular respiration were identified. These included the Ferredoxin *YAH1* that functions in heme A biosynthesis [19]. The GMP synthase *GUA1* that has a role in biosynthesis of GMP from inosine 5′-phosphate [20]. The transmembrane protein EMP70 that is involved in endosome-to-vacuole sorting [21]. The cellular transported PDR15 that is involved with cellular detoxification [22]. Overall the data presented here indicates that RNAseq analysis using the Ion Torrent PGM yielded similar results to those obtained using Afflymetrix microarrays.

Discussion

We found that yeast cells cultured in a high glucose environment exhibited a change from fermentation to respiration during aerobic growth evidenced by the fact that the amount of ethanol produced became very minimal. Through whole genome resequencing of three isolated strains, several non-synonymous mutations were identified, which further support

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our phenotypic change. RNAseq and microarray analysis identified mRNAs that were differentially regulated between the parental strain and the three evolved strains. These mRNAs are involved in the TCA cycle and aerobic respiration. To conclude, the data presented here indicates that the yeast strain *S. cerevisiae* has the ability to undergo adaptive evolution through natural selection. This work clearly highlights the importance of adaptive evolution and its potential role in ensuring cell survival, specifically under adverse growth or environmental conditions.

There have been several attempts to develop a yeast strain that prefers respiration rather than fermentation in the presence of high glucose concentrations [23-27]. However, our study is different in that we selected directly for a yeast strain that grows optimally (or maximally) when glucose is non-limiting. Therefore, we performed our selection of the yeast cells in constant maximal exponential growth phase by diluting a nutrient rich batch culture. Due to the selection we performed, the optimal metabolic activity of the yeast should be directly comparable to simulation results using Flux Balance Analysis (FBA) [28-32]. Overall, our measured phenotypic changes, changes in the gene expression, and changes in the genome support the shift in metabolism toward optimal behavior. In the future, metabolomics studies could be performed to determine if the changes in the metabolic fluxes are consistent with FBA results.

Finally, the evolutionary selection we performed can be used as a method for determining the integrated function of cellular networks. For example, determining the mechanism that leads to the observed changes in phenotype will likely shed light on the operation of the networks. First, the phenotype is highly correlated with the observed changes in gene expression. The next question is how do the observed changes in gene expression correlate with the changes in the genome? Our results indicated that mutations in regulators may play an important role, but the next important question involves finding the regulatory pathways involved. This study and future similar studies will be very useful for uncovering the systems biology of yeast or other organisms.

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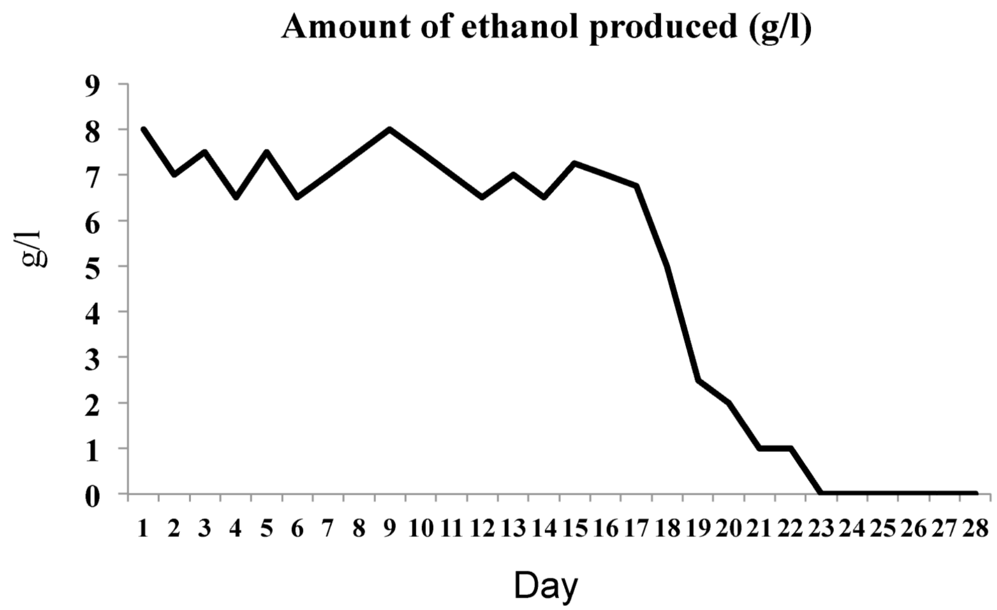

Fig1.

Measurements of the amount (g/l) of ethanol produced during aerobic of a batch culture while growing in high glucose.

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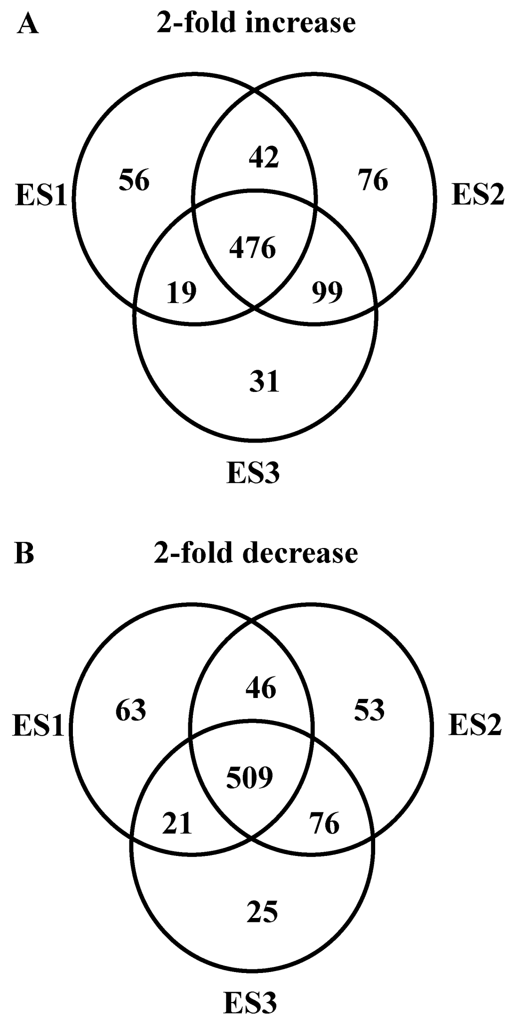

Fig2.

Venn diagram of transcripts that increased (A) or decreased (B) in abundance in the evolved strains relative to the parental (BY4741) after 450 generations in a high glucose environment.

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Table 1

DIPs detected in protein coating genes using clc bio workbench

Gene	Amino Acid Change	Gene Function[1]
SCW11	Pro441fs	Cell wall protein
NOP7	Leu584fs	Component of several different pre-ribosomal particles
YDR261C-D	Lys1396fs	DNA-directed DNA polymerase activity
UTP20	Leu1232fs	Involved in the biogenesis of the 18S rRNA
YSW1	Glu451_Lys452delinsGlu	Protein required for normal prospore membrane formation
NUM1	Glu857fs	Protein required for nuclear migration
YDR387C	Ser378fs	Putative transporter
SLM3	Pro70fs	Responsible for 2-thiolation of the wobble base of mitochondrial tRNAs
ITC1	Lys818fs	Subunit of the ATP-dependent Isw2p-Itc1p chromatin remodeling complex
KRS1	Asp379fs	Aminoacyl-tRNA synthetase specific for lysine
YDL129W	Val278fs	Unknown
YDR018C	Asn395fs	Unknown
PHO8	Arg411fs	vacuolar alkaline phosphatas
DGR2	Lys132fs	Unknown
YCF1	Met287fs	Vacuolar glutathion S-Conjugate transporter
COS2	Arg50fs	Unknown
SRL2	Ser24fs	Unknown
YPL279C	Lev121fs	Unknown

[1] Saccharomyces Genome Database

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Table 2

SNPs detected in protein coating genes using clc bio workbench

Gene	Amino Acid Change	Gene Function[1]
RRT12	Gly262Val	A role in formation of the dityrosine layer of spore walls
ALY2	Trp112Arg	Alpha arrestin
RBG1	Ile220Thr	Associates with translating ribosomes
SMC2	Phe509Cys	AT DNA binding
CDC48	Ala684Glu	ATPase involved in ubiquitin-mediated protein degradation
PRP16	Ala875Val	DEAH-box RNA helicase involved in second catalytic step of splicing
VRG4	Val163Glu	Golgi GDP-mannose transporter
HXT2	Thr186Ala	High-affinity glucose transporter of the major facilitator superfamily
HDA2	Asn647Lys	Histone DeAcetylase
SRD1	Glu97Lys	Involved in the processing of pre-rRNA to mature rRNA
GRX7	Ser202Thr	Oxidative stress response
TYR1	Asp379Gly	Prephenate dehydrogenase involved in tyrosine biosynthesis
RPS26A	Lys66Asn	Protein component of the small (40S) ribosomal subunit
PKC1	Ile866Leu	Protein serine/threonine kinase
NAP1	Phe264Leu	Protein that interacts with mitotic cyclin Clb2p
MDS3	Asn1329Lys	Putative component of the TOR regulatory pathway
YBP1	Val233Ala	Oxidation of specific cysteine residues of the transcription factor Yap1p
VPS75	Gly246Ser	Stimulates histone acetyltransferase activity
APC2	Ile845Val	Subunit of the Anaphase-Promoting Complex/Cyclosome
NPL4	Ser378Phe	Ubiquitin-binding protein involved in protein degradation
UBP3	Pro407Ser	Ubiquitin-specific protease
VPS41	Asn928Ser	Vacuolar membrane protein
YPR114w	Ser110Gly	Unknown
YPL260w	Met241Lys	Putative substrate of cAMP-dependent protein kinae (PKA)
YJR061w	Phe555Leu	Unknown
TBS1	Asn1013Asp	Unknown

[1] Saccharomyces Genome Database

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Table 3

Significant gene expression changes in gene ontology categories determined using GSEA

Description	Size	Test statistic
Up-regulated in evolved strains relative to parental		
tricarboxylic acid cycle	28	−8.28
aerobic respiration	58	−6.71
regulation of transcription, DNA-dependent	511	−6.25
mitochondrial electron transport, ubiquinol to cytochrome c	11	−5.45
mitochondrial translation	108	−5.42
transcription, DNA-dependent	527	−5.37
electron transport chain	48	−5.12
protein processing	15	−4.94
signal transduction	73	−4.87
response to stress	150	−4.68
thiamin biosynthetic process	17	−4.61
protein catabolic process	17	−4.43
proteasomal ubiquitin-dependent protein catabolic process	33	−4.42
mitochondrion organization	36	−4.31
cellular carbohydrate metabolic process	17	−4.27
Down-regulated in evolved strains relative to parental		
arginine biosynthetic process	10	5.01
Gluconeogenesis	18	5.09
tRNA aminoacylation for protein translation	35	5.10
aromatic amino acid family biosynthetic process	12	5.75
maturation of SSU-rRNA from tricistronic rRNA transcript	61	6.42
cellular amino acid biosynthetic process	98	7.02
rRNA export from nucleus	27	7.35
Glycolysis	25	7.67
Translation	241	10.33