Metabolite profiles indicative of accelerated aging phenotypes in succinate dehydrogenase mutants in *S. cerevisiae*

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Introduction

Yeast chronological lifespan (**CLS**) is measured by the age of yeast cultures after entry to stationary phase *vs.* the percentage of cells capable of colony formation upon exposure to fresh media. We use quantitative high throughput cell array phenotyping (**Q-HTCP**) for growth curve analysis of isogenic yeast gene knockout strains to understand the influences of and interactions between genetic components upon chronological aging. Among many genetic effects on CLS, we observed that mutants in the succinate dehydrogenase (SDH) complex have reduced CLS (i.e. accelerated chronological aging). To understand the role of SDH in aging from a metabolomic perspective, we performed a high resolution experiment involving measurement of over 400 metabolite ions at 11 time points from aging yeast cultures of the wild type (wt) and mutants in the four structural subunits of SDH.

Background

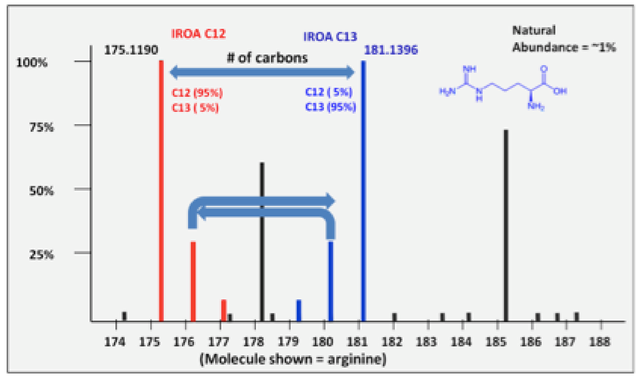
- Yeast (*Saccharomyces cerevisiae*) is a highly annotated genetic model organism widely adopted to study aging.
- SDH is a mitochondrial enzyme comprised of four subunits (SDH1-4 in yeast) and mutations in the human SDH are linked to both mitochondrial disease and cancer.
- The yeast chronological aging model and IROA protocols were used to develop a Q-HTCP procedure to investigate the metabolic effects of defective SDH activity through deletion of the SDH 1, 2 and 4 subunits, and knockdown of the SDH3 subunit using a sdh3 DAmP strain.

Strains, Media, and Reagents

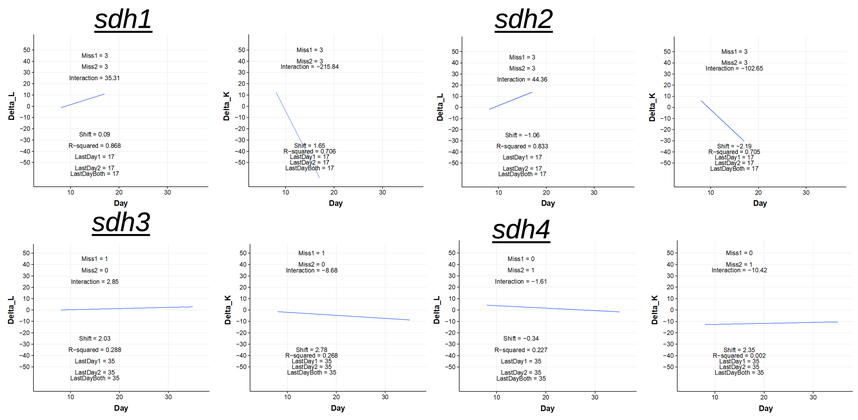
- Yeast were BY4741a (*his3 leu2 met17 ura3*) background including the *sdh1, sdh2, and sdh4* strains from the commercially available gene deletion strain collection and the *sdh3* DAmP strain.
- HLD media with 2% glucose (unbuffered, without ammonium sulfate, see PMID 25668739).
- IROA 95% ^{13}C-labeled yeast extract (Cambridge Isotope Laboratories).

Methods

- Quantitative High Throughput Cell Array Phenotyping was performed as previously described.
- Cultures were aged for metabolomic analysis in single shaking flasks at 30 ºC.
- Metabolite extractions were performed after vacuum filtration of 5 mL of culture, using 80% methanol with chloroform at -80 ºC (dry ice / ETOH), vortexing every 5 minutes for 30 minutes.
- Metabolite extracts were dried down, resuspended with IROA internal standards, and re-dried.
- The phenotypic IROA method (IROA Technologies) was performed using the IROA WORKFLOW Kit with 95% ^{13}C-labeled yeast extract (Cambridge Isotope Laboratories).
- Mass spectrometry was performed at the Southeast Center for Integrated Metabolomic (SECIM) on a Thermo Q Exactive instrument with resolution of 35,000 at m/z 200, using reverse phase UHLC-HRMS with a C18-pentafluorophenyl (PFP) column.
- Mass spectrometry files were converted to mzxml using ProteoWizard.
- ClusterFinder Software (IROA Technologies) was used for analysis of converted files.
- The Discovery software and Mass Spectrometry Metabolite Library of Standards (MSMLS; IROA Technologies) was used to identify specific metabolites (targeted analysis).
- Custom MatLab scripts were developed to plot the metabolite profiles output from ClusterFinder.
- Aging metabolite profiles were visually inspected and classified.
- The statistics modules of Metaboanalyst and ClusterFinder were used to characterize
- results.



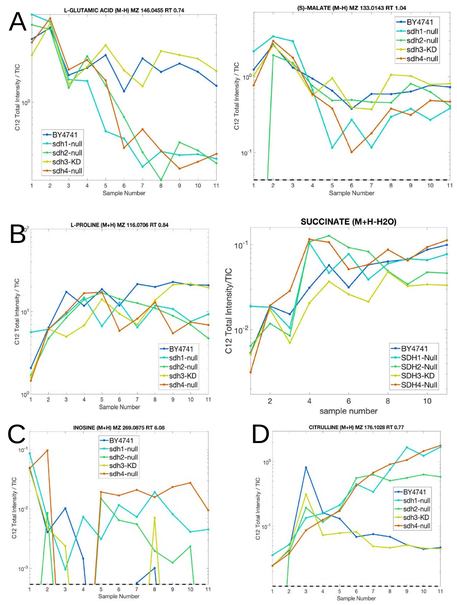
Isotopic Ratio Outlier Analysis (IROA) to identify metabolites by ^{13}C isotopic labeling. Using the IROA approach, the number of carbons from each ion can be inferred by the 'envelope' of associated isotopic peaks. The number of carbons is revealed by both the distance between the fully C12 and C13 –incorporated isotopic peaks, and the relative peak heights of adjacent isotopic peaks. Using the number of carbons, together with the exact mass of each ion, increases the accuracy of chemical formula prediction.

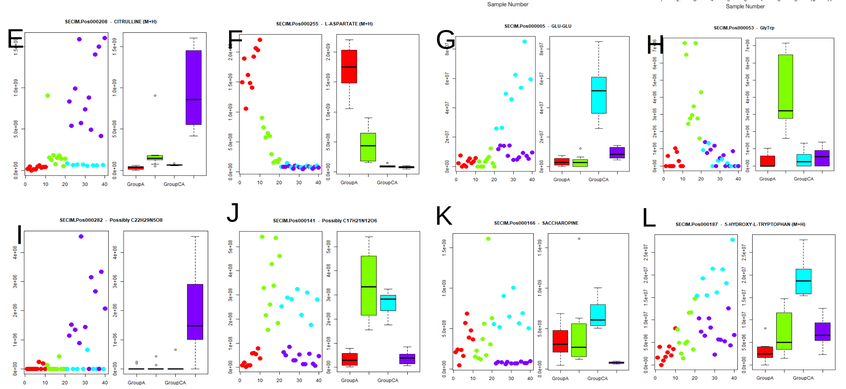


Succinate dehydrogenase mutants have reduced CLS. Data from a genome-wide CLS screen are shown. Duplicate cultures for each sdh mutant are shown against the distribution of CLS phenotypes for the non-mutant parental strain.

Time Point	Day	Hours
1	1	16.5
2	1	22.5
3	1	26
4	2	38.5
5	2	45.5
6	3	62.5
7	3	69.5
8	4	88.5
9	5	119.5
10	6	137
11	7	158.5

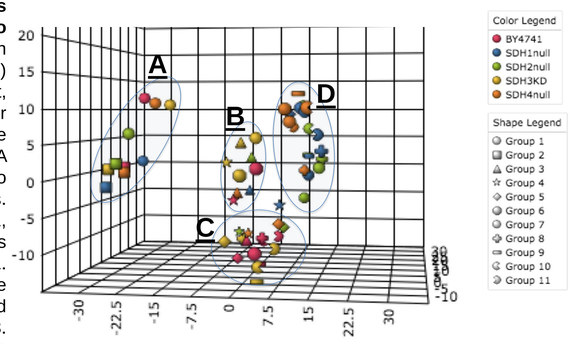
Table 1. CLS time points for metabolite extractions





Long and short-lived yeast exhibit distinct metabolite profiles. **(A-D)** Temporal patterns of metabolite expression included: **(A)** reducing or **(B)** increasing pools with age, **(C)** initial reduction followed by an increase, and **(D)** initial increase with subsequent reduction. The selected examples compare expression between strains having longer (wt/BY4741 and sdh3) *vs.* shorter (sdh1/sdh2/sdh4) chronological lifespan. **(E-L)** As another way of visualizing metabolites differentially expressed with age and correlations with chronological aging phenotypes, we grouped the samples according to age and mutant status for statistical comparisons. The four groups shown are: (RED) = time points 1 and 2 for all strains; (GREEN) = time points 3 and 4 for all strains; (BLUE) = time points 8-11 for wild type and *sdh3* (mild hypomorphic allele with normal longevity); (PURPLE) = time points 8-11 for *sdh1*, *sdh2*, and *sdh4* mutants. **(E)** Result for citrulline, alongside the graphed data (panel D). Additional types of differential expression were observed including: **(F)** decreasing with age, similarly expressed in long and short-lived strains; **(G)** increasing with age, positively correlated with longevity; **(H)** transiently increased, not correlated with longevity; **(I)** expressed only in the accelerated aging condition; **(J)** increasing expression maintained only with normal longevity; **(K-L)** additional examples with subtle distinction from those described above.

Metabolite extracts cluster according to age and strain. Each Group (shape legend) represents a time point, listed in Table 1. Four distinct Clusters are highlighted. Cluster A consists of the first two time points for all strains. Cluster D contains sdh1, sdh2, and sdh4 samples for time points 6-11. Cluster C contains all late time points (8-11) for wild type and sdh3. Intermediate time points for all strains occur mostly in Clusters B or C.



Metabolite	down	up	up-down	down up	wt/3 split	other split
'5-HYDROXY-L-TRYPTOPHAN (M+H) MZ 221.0921 RT 6.68'		X			X	
'5-OXO-D-PROLINE (M+Na) MZ 152.0318 RT 1.75'		X				
'ADENOSINE (M+H) MZ 268.1042 RT 5.67'		X				X
'CARNOSINE (M+H) MZ 227.1141 RT 0.7'						X
'CITRULLINE (M+H) MZ 176.1028 RT 0.77'			X		X	
'D-ASPARTATE (M+H) MZ 134.0446 RT 0.7'	X					
'D-LACTOSE (M+H-H2O) MZ 325.1117 RT 0.76'			X			X
'D-TRYPTOPHAN (M+H) MZ 205.0968 RT 7.66'			X			X
'GLYCEROL (M+H) MZ 93.0547 RT 0.78'						
'GUANOSINE (M+H) MZ 284.0984 RT 6.22'		X				X
'HOMOSERINE (M+H) MZ 120.0656 RT 0.72'	X					X
'HYPOXANTHINE (M+H) MZ 137.0459 RT 1.96'	X				X	X
'INOSINE (M+H) MZ 269.0875 RT 6.08'				X	X	
'L-GLUTAMIC ACID (M+H) MZ 148.0605 RT 0.75'	X				X	
'L-GLUTAMINE (M+H) MZ 147.0764 RT 0.72'	X					X
'L-HISTIDINE (M+K) MZ 194.0329 RT 0.68'		X				
'L-LYSINE (M+H) MZ 147.1128 RT 0.89'			X			X
'L-METHIONINE (M+H) MZ 150.0585 RT 1.38'						
'L-PHENYLALANINE (M+H) MZ 166.0863 RT 6.29'						
'L-PROLINE (M+H) MZ 116.0706 RT 0.84'		X			X	
'L-SERINE (M+H) MZ 106.0499 RT 0.69'						
'L-TYROSINE (M+H) MZ 182.0812 RT 3.35'		X				X
'MELIBIOSE (M+NH4) MZ 360.15 RT 0.78'			X			X
'N-ACETYL-DL-GLUTAMIC ACID (M+H) MZ 190.0711 RT 2.13'			X		X	
'N-ACETYLNEURAMINATE (M+H) MZ 310.1123 RT 0.8'	X					
'NEPSILON,NEPSILON,NEPSILON-TRIMETHYLLYSINE (M+H) MZ 189.15	X				X	
'NICOTINAMIDE (M+H) MZ 123.0553 RT 1.57'				X		
'NICOTINATE (M+H) MZ 124.0394 RT 1.31'				X		
'SARCOSINE (M+H) MZ 90.0551 RT 0.72'			X			
'SUCCINATE (M+H-H2O) MZ 101.0234 RT 2.23'		X				
'URACIL (M+H) MZ 113.0347 RT 1.34'						
'URIDINE (M+H) MZ 245.0766 RT 2.73'		X				

Table 2. Temporal expression pattern and differential expression for metabolites identified from the MSMLS.

Summary

- The yeast chronological aging model was applied to understand how metabolism changes with age and to identify metabolites potentially associated with aging.
- Metabolites were extracted from five yeast cultures at eleven time points over 7 days, the window of time when metabolic effects on yeast longevity occur.
- IROA was performed to systematically assess metabolite pools. MSMLS was used for large-scale targeted analysis, identifying ~ 50 metabolites. An additional 300+ metabolites were detected.
- Mutants in succinate dehydrogenase were found to have reduced CLS from a genome-wide screen of the yeast gene deletion mutants.
- PCA analysis revealed age-related differential expression for most metabolites and aging phenotype-associated correlations for several metabolite profiles.
- All metabolites were graphed and scored to understand age-related expression and aging phenotype correlations.
- For statistical analysis, yeast cultures were grouped according to their lifespan; sdh1, 2, and 4 knockouts having reduced CLS and wt and sdh3 normal longevity.
- Numerous metabolites were differentially expressed with age, and additionally correlated with the chronological aging phenotype.
- Future direction is to integrate this and additional related information to describe how metabolism influences aging.

Acknowledgements

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• Contact jhartman@uab.edu with any questions