Phenomic analysis of the influence and interactions of auxotrophy and nutrient availability on yeast quiescence and chronological lifespan

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ABSTRACT

Yeast cells in stationary phase culture exhibit different developmental fates, including death, senescence, and quiescence. Quiescence is an adaptive transition to a stable cell cycle exit (G0, prior to G1-S transition) in response to nutrient depletion in stationary phase, which is defined functionally by colony forming capacity upon re-exposure to nutritive conditions. Thus, quiescence vs. time constitutes yeast chronological lifespan (CLS). Results from high-throughput CLS studies only weakly correlate, which could be partly due to influences of media composition and auxotrophy on quiescence. This hypothesis was investigated with quantitative high-throughput cell array phenotyping (Q-HTCP) to measure impacts of auxotrophy and media composition on quiescence / CLS, characterizing quiescence over longer-than-typical time periods (past 30 days), and in greater replicate so that high-resolution phenotypic distributions are obtained for aging interventions. In these regards, auxotrophic alleles in the genetic background of the S288C gene deletion library (*his3, leu2, lys2, met17, ura3*), along with glucose, ammonium sulfate, auxotrophic amino acid availability were systematically studied. Target of Rapamycin signaling and replication stress were also examined in this context. Media acidification was monitored in high throughput to assess its correlation with CLS. Previously reported influences of leucine, methionine or glucose availability were observed, and a novel effect of lysine auxotrophy characterized. By contrast, histidine and uracil perturbations had little or no effect on quiescence. Ammonium sulfate availability altered CLS, but this affect was dependent on TOR1 expression and methionine metabolism. The pH of conditioned media from aged cultures was dependent on interactions between auxotrophy and aeration; however, there was weak correlation between media acidification and CLS. In summary, interacting effects of auxotrophy, media composition, and aeration on yeast cell quiescence indicate a fundamental role of metabolism in yeast chronological aging. These factors interact and differentially influence cell quiescence or the pH of conditioned media, however CLS and media acidification are weakly correlated. Hormesis-like effects were sometimes observed, whereby early loss of CFU capacity was associated with later preservation. Gasping, defined as a transient increase in CFU capacity in stationary phase, was observed to be largely independent of establishment or maintenance of quiescence. Quiescence was typically dynamic in the first month, followed by either stable establishment or complete loss. Taken together, these results suggest CLS is a developmental process that is more complex than previously considered and highlight parameters that can potentially explain variability in high throughput studies of CLS.

Methods

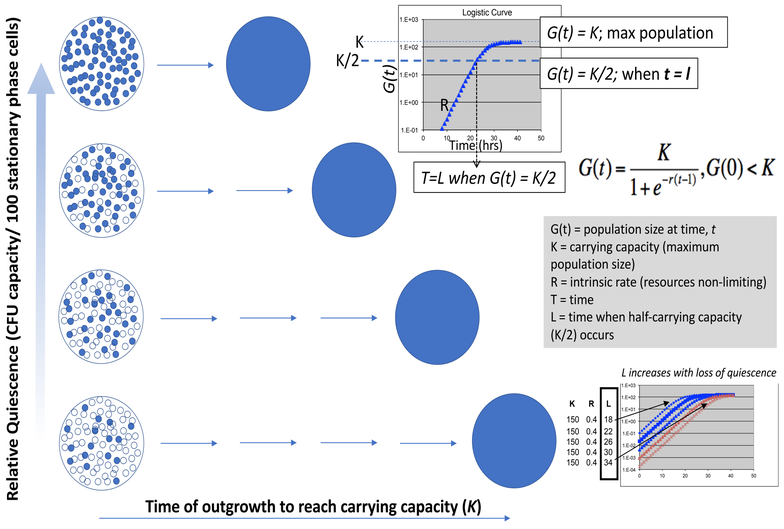


Figure 1. Q-HTCP method for quiescence profiling

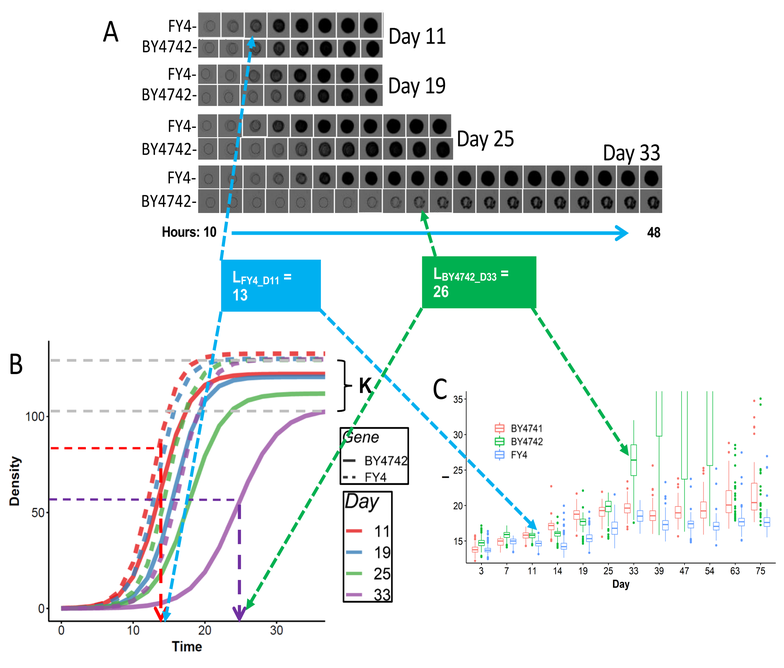
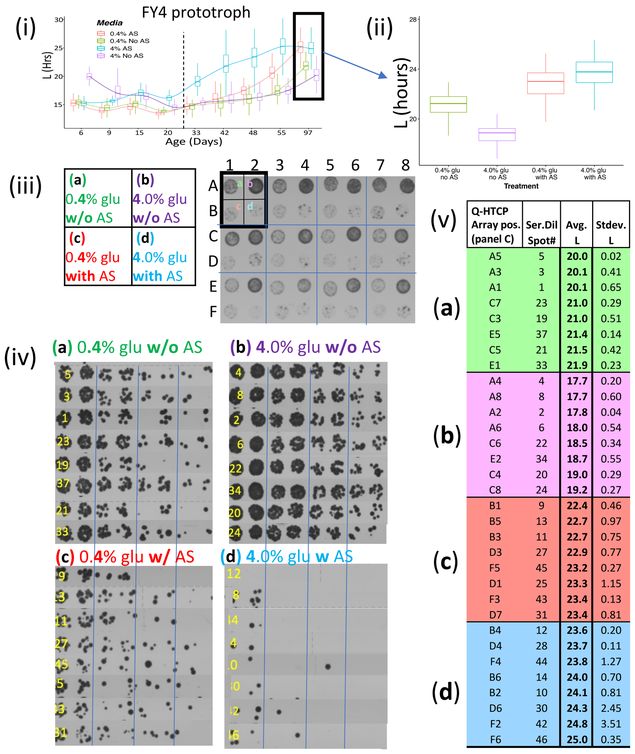


Figure 2. Quiescence profiling example – FY4 prototrophic strain, BY4741 and BY4742

Results



	Q-HTCP Array pos. (panel C)	Ser.Dil Spot#	Avg. L	Stdev. L
(a)	A5	5	20.0	0.02
	A3	3	20.1	0.41
	A1	1	20.1	0.65
	C7	23	21.0	0.29
	C3	19	21.0	0.51
	E5	37	21.4	0.14
	C5	21	21.5	0.42
	E1	33	21.9	0.23
(b)	A4	4	17.7	0.20
	A8	8	17.7	0.60
	A2	2	17.8	0.04
	A6	6	18.0	0.54
	C6	22	18.5	0.34
	E2	34	18.7	0.55
	C4	20	19.0	0.29
	C8	24	19.2	0.27
(c)	B1	9	22.4	0.46
	B5	13	22.7	0.97
	B3	11	22.7	0.75
	D3	27	22.9	0.77
	F5	45	23.2	0.27
	D1	25	23.3	1.15
	F3	43	23.4	0.13
	D7	31	23.4	0.81
(d)	B4	12	23.6	0.20
	D4	28	23.7	0.11
	F4	44	23.8	1.27
	B6	14	24.0	0.70
	B2	10	24.1	0.81
	D6	30	24.3	2.45
	F2	42	24.8	3.51
	F6	46	25.0	0.35

Figure 3. Relationship of the growth parameter, L to serial dilution spot test for assessing colony forming capacity of stationary phase culture.

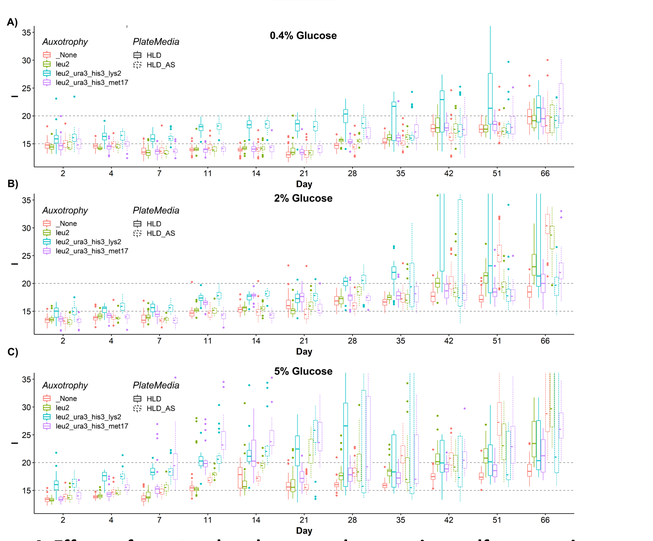


Figure 4. Effects of auxotrophy, glucose and ammonium sulfate on quiescence.

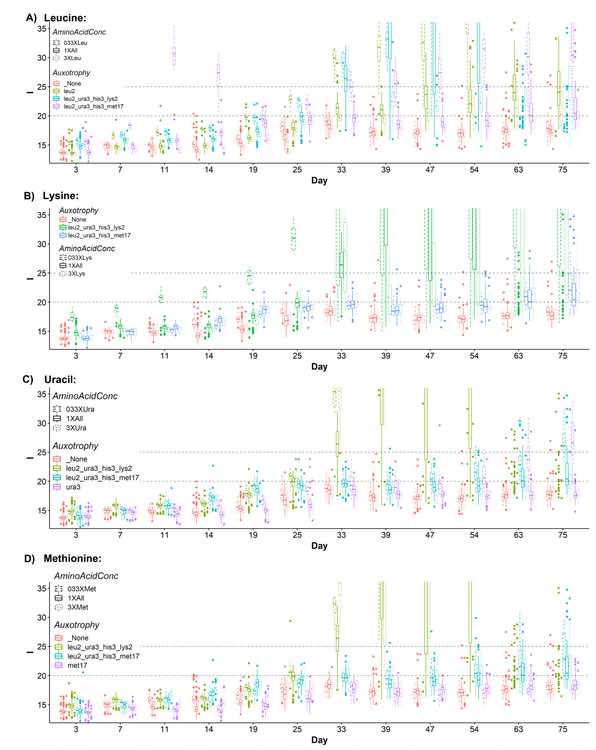


Figure 5. Effects of specific nutrient limitation and auxotrophy, on quiescence.

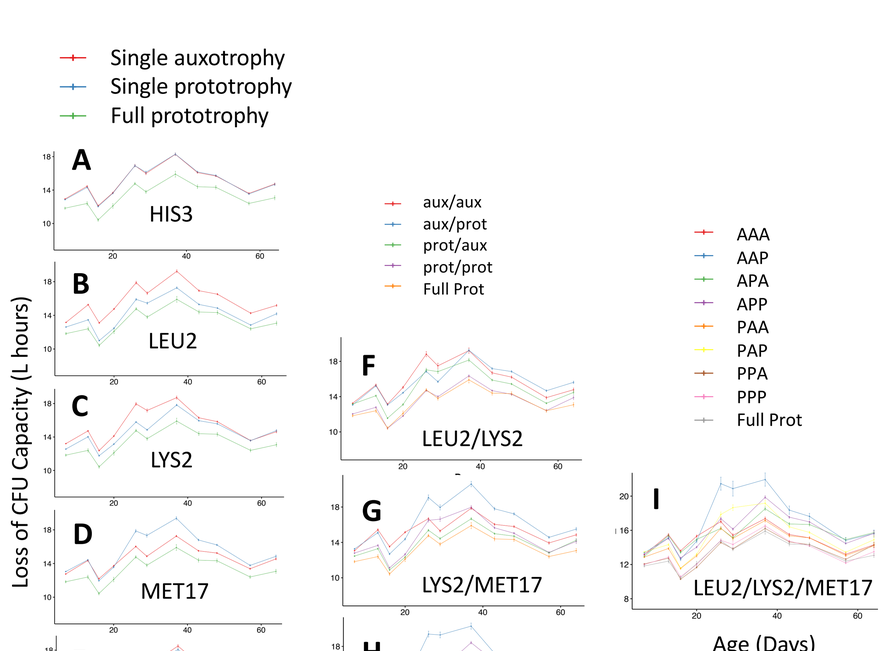


Figure 6. Interactions between auxotrophic loci influence quiescence. "A" and "P" in the legends refer to auxotrophy and prototrophy of loci, as ordered on the graph labels. (A-E) single locus effects; (F-H) pairwise interactions; (I) 3- way interactions.

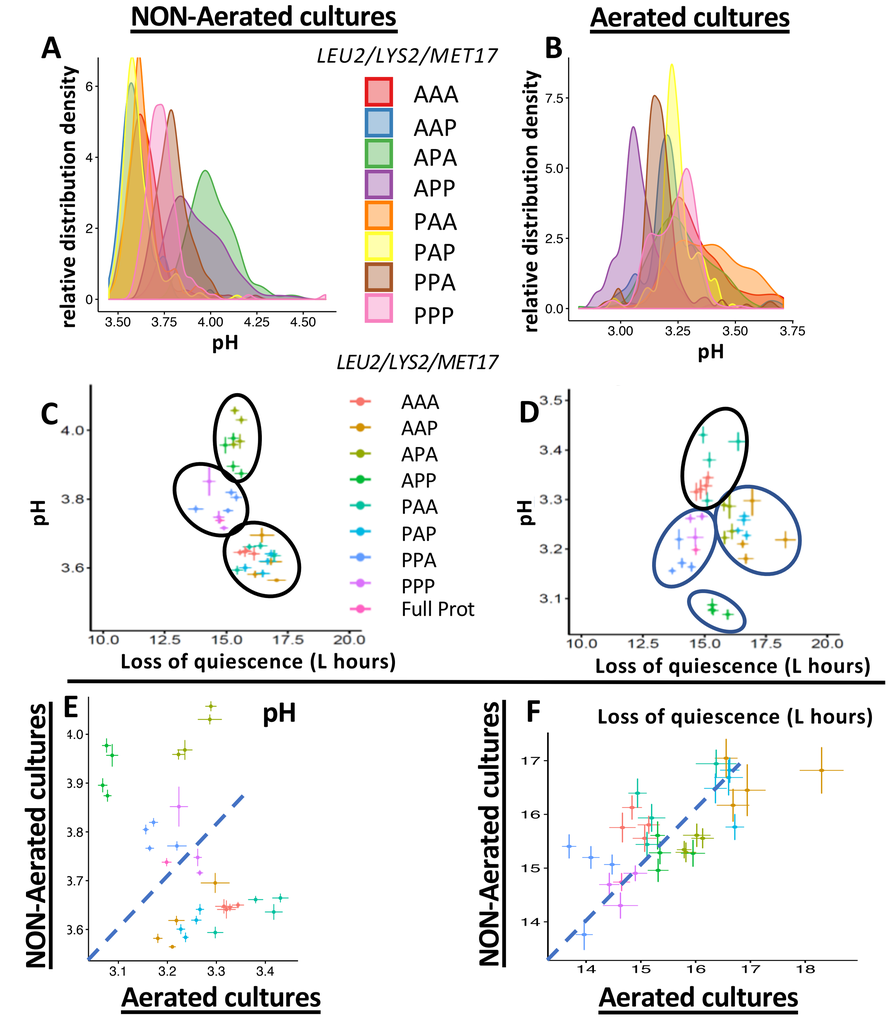


Figure 7. Interactions between auxotrophic loci influence quiescence. "A" and "P" in the legends refer to auxotrophy and prototrophy of loci, as ordered on the graph labels. (A-E) single locus effects; (F-H) pairwise interactions; (I) 3- way interactions.

Conclusion and future directions: (1) Quantitative high throughput cell array phenotyping (Q-HTCP) is a powerful method for quiescence profiling; (2) HL media may provide a nutrient context of broader relevance to quiescence in other eukaryotes; (3) auxotrophy and media composition strongly influence quiescence such that genome-wide analysis in a prototrophic context may be additionally informative to past studies; (4) media acidification and quiescence are only weakly correlated in HL media.

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