Buffering the pH of the culture medium does not extend yeast replicative lifespan [version 1; peer review: 2 approved]

Brian M Wasko, Daniel T Carr, Herman Tung, Ha Doan, Nathan Schurman, Jillian R Neault, Joey Feng, Janet Lee, Ben Zipkin, Jacob Mouser, Edward Oudanonh, Tina Nguyen, Torin Stetina, Anna Shemorry, Mekedes Lemma, Matt Kaeberlein

Department of Pathology, University of Washington, Seattle, WA, 98195, USA

Open Peer Review

Approval Status ✓ ✓

version 1 15 Oct 2013 ✓ view ✓ view

1. Karim Mekhail, University of Toronto, Toronto, Canada
2. Cory Dunn, Koç University, Istanbul, Turkey

Any reports and responses or comments on the article can be found at the end of the article.

Abstract

During chronological aging of budding yeast cells, the culture medium can become acidified, and this acidification limits cell survival. As a consequence, buffering the culture medium to pH 6 significantly extends chronological life span under standard conditions in synthetic medium. In this study, we assessed whether a similar process occurs during replicative aging of yeast cells. We find no evidence that buffering the pH of the culture medium to pH levels either higher or lower than the initial pH of the medium is able to significantly extend replicative lifespan. Thus, we conclude that, unlike chronological life span, replicative life span is not limited by acidification of the culture medium or by changes in the pH of the environment.
Introduction

Aging has been studied extensively in the budding yeast Saccharomyces cerevisiae using two fundamentally different systems: the replicative lifespan assay and the chronological lifespan assay. Replicative life span is defined as the number of daughter cells that a mother cell can produce prior to entering an irreversible cell cycle arrest, while chronological lifespan is defined as the length of time that a yeast cell can maintain viability in a non-dividing state. Numerous genetic and environmental factors have been identified that can modulate either replicative aging, or chronological aging, or both.

Replicative aging has been studied almost exclusively by maintaining individual cells on the surface of a nutrient agar plate, microdissecting daughter cells away from the mother cells, and counting the number of daughter cells that the mother cell produces prior to senescence. Generally, rich YPD medium (2% glucose) is used for replicative lifespan assays. Calorie restriction by reducing the glucose concentration of the medium to 0.5% or lower has been shown in numerous studies to extend lifespan in different wild type strain backgrounds between 10–40%.

Several methods have been described for studying chronological aging. The most widely utilized protocol involves culturing yeast cells in synthetic complete liquid medium with 2% glucose as the carbon source, either under shaking or static conditions, in culture tubes or 96-well plates. Alternative, but less frequently used, liquid culture methods for chronological aging involve culturing cells in rich YPD medium, using a respiratory carbon source such as glycerol, or transferring cells to water once they have reached stationary phase growth arrest. A plate-based assay for chronological life span analysis has also been described in which cells are growth arrested through limitation for tryptophan. In all of these assays, viability over time is determined by restoring a small subset of the population to nutrient rich growth conditions and assaying their ability to re-enter the cell cycle, either through quantification of colony forming units on solid-agar plates or through outgrowth kinetics in liquid culture. Similar to the case for replicative lifespan, calorie restriction by reducing the initial glucose concentration of the culture medium can extend chronological lifespan, generally by more than 100%.

One important feature of the standard method for determining chronological aging is that the culture medium becomes acidified over the first few days of the experiment, with pH dropping from an initial value of around 4.0 to 2.5–2.9 within 96 hours. This acidification of the external environment results from the production of organic acids, including acetic acid, following fermentation of glucose to ethanol and subsequent utilization of ethanol as a carbon source once the glucose is depleted. Preventing medium acidification by buffering the culture to a pH of 6.0 with either citrate phosphate buffer or low salt MES buffer results in a more than doubling of chronological lifespan. Calorie restriction, or switching the yeast culture to a non-fermentable carbon source, such as glycerol or ethanol, also prevents acidification and results in a similar magnitude of chronological lifespan extension as buffering.

Although the two yeast aging assays are nearly always studied independently, it is clear that they share at least some overlap. As mentioned above, calorie restriction extends both replicative and chronological lifespan, as do a few genetic interventions, such as deletion of either TOR1 or SCH9, both of which are nutrient-responsive kinases. In addition, it has been shown that chronologically aged cells have reduced replicative lifespan when returned to rich growth conditions. This reduction in replicative lifespan following chronological aging appears to be mediated through changes in mitochondrial function, since the chronologically old cells that retain the lowest mitochondrial membrane potential also have the longest replicative lifespan following resumption of cell division. Calorie restriction or buffering the culture medium of the cells during chronological aging also protects against subsequent replicative lifespan reduction, raising the possibility that medium acidification directly influences both types of yeast aging. To assess this possibility, we performed replicative lifespan analysis on wild type BY4742 mother cells under either standard conditions or on rich media buffered to different pH values. We were unable to detect a significant replicative lifespan extension from buffering the culture medium under any of the conditions examined, including those conditions that robustly extend chronological lifespan.

Methods

Reproductive lifespans

All lifespan experiments were performed in the BY4742 strain background (Thermo Scientific, Waltham, MA) as previously described. Virgin daughter cells were isolated and allowed to grow into mother cells while their corresponding daughters were micro-dissected using Zeiss Axioskop 40 dissection microscopes and manually counted until the mother cell could no longer divide. YEP agar plates (1% yeast extract, 2% bacto-peptone, 2% agar) containing 2% glucose (YPD) were utilized and strains were grown at 30°C during the day, dissected at room temperature, and placed in a refrigerator at 4°C over night. Daughter cells were removed from each mother cell roughly every 2 hours by micromanipulation. Cells were scored as senescent when they had failed to divide for at least eight hours of incubation at 30°C. Terminal morphology was defined as the budding state of the mother cells upon senescence. All experiments were performed by a team of dissectors who were blinded to the identity of the strains under examination in any given experiment. Prism Graphpad 5.0 was used for data analysis. Statistical significance for differences in median lifespan was determined using the Wilcoxon Rank-Sum test. Budded and unbudded states were determined visually for each mother cell assayed and statistical comparisons of budding rates utilized Fischer’s Exact two-tailed test. Multiple comparison corrections were performed using the Bonferroni correction.

Preparation of media

Stock buffers were prepared at 1 M in deionized water and pH was adjusted by addition of appropriate molar ratios of conjugate acid and conjugate base, or by empirical adjustment with HCl or NaOH. Buffer reagents were obtained from Sigma-Aldrich (St Louis, MO). The following buffers were used: Tris(hydroxymethyl)aminomethane (Tris) ; 3-(N-morpholino)propanesulfonic acid (MOPS); 2-(N-morpholino)ethanesulfonic acid (MES); citrate buffer (sodium citrate and citric acid); acetate buffer (sodium acetate and acetic acid). Stock buffers were sterilized by filtration using a VWR International syringe driven 0.2 micron cellulose acetate membrane filter. Buffers were diluted to
100 mM final concentration in YPD agar after autoclaving and cooling to ~55°C. Appropriate stock buffer pH was empirically determined, as necessary to adjust the pH of YPD liquid media at room temperature to the indicated final pH. Measurement of pH was performed using an Accumet Excel XL15 pH meter. The pH of agar media was further verified by use of EMD colorPHas pH indicator strips. For adjustment of media pH without buffer addition, the indicated acid or base was added to achieve the desired pH prior to autoclaving.

**Results**

**pH and buffering**

Lowering the pH of YPD agar plates to 5.0 by HCl (p = 0.0218) and to 6.0 using MES buffer (p = 0.0165) trended toward a decrease in lifespan that was statistically significant without adjusting for multiple comparisons (α = 0.05), but which did not reach significance after adjusting for multiple comparisons using the Bonferroni correction (α = 0.0056) (Figure 1A, Table 1). Lowering pH to 5.0 by acetic acid (p = 0.8869) or buffering to pH 5.6 using acetate buffer (p = 0.0896) had no detectable effect on lifespan. Further reduction of pH to 3.0 using a citrate buffer (p = 0.3412) also had no effect on lifespan. Buffering YPD media to pH 7 by MOPS (p = 0.4161) or Tris (p = 0.3653) had no detectable effect on yeast lifespan (Figure 1B). Increasing the pH of YPD to 8.0 by NaOH (p = 0.7658) or buffering at pH 7.8 with 100 mM Tris (p = 0.1754) also had no detectable effect on lifespan. Raising pH further to 9.0 by sodium hydroxide (p < 0.0001) resulted in a significant reduction of lifespan (Figure 1C), and cells on YPD buffered to pH 9.0 with 100 mM Tris buffer did not divide and, thus, replicative lifespan could not be determined for this condition.

**Terminal morphology**

Terminal morphology is defined as the budded state of the mother cells upon senescence23. Terminal morphology frequency was not significantly altered when pH was buffered at 7.0 by MOPS (p = 0.8224) or 7.0 (p = 0.8224) or 7.8 by Tris (p = 1) or by adjustment of pH to 8.0 by sodium hydroxide (p = 0.3711). Manipulations that lowered the pH displayed a trend toward a higher percentage of unbudded cells upon arrest (Table 1), but this did not reach statistical significance after correcting for multiple testing (α = 0.0056).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Replicative lifespans with pH adjusted to 7–8 by buffering or NaOH (A), or pH 6.0 or below by acids or buffers (B) and to pH 9.0 by NaOH (C).
Discussion

The results presented here demonstrate that, unlike chronological lifespan, acidification of the culture medium does not limit replicative lifespan under standard conditions. This is relevant information, because it rules out the possibility that interventions shown to extend replicative lifespan are acting by either reducing the production and secretion of organic acids into the environment or by increasing resistance to acid stress.

The biological relevance of acidification limiting chronological lifespan has been an area of contention within the field, due in part to concerns that cell death due to acidification may be a yeast specific phenomenon. Evidence supporting this concern has been provided by parallel analyses of replicative and chronological lifespan for yeast deletion mutants corresponding to *Caenorhabditis elegans* genes that increase lifespan when their expression is reduced. A significant enrichment for long replicative lifespan was found among this set of yeast deletions, but no enrichment for increased chronological lifespan under standard conditions was observed. On the other hand, there is evidence that a similar acid-induced mechanism of senescence occurs in mammalian cells, at least in culture, suggesting the possibility that the intracellular response to external pH may be conserved.

The trend toward reduced lifespan noted under some of the conditions tested is of interest and may warrant further study. The significant reduction in lifespan associated with adjusting to pH 9.0 by NaOH may reflect a reduced ability of yeast to proliferate under basic conditions, which is consistent with the inability of yeast cells to grow in the replicative lifespan assay when the YPD was buffered to pH 9.0 by Tris buffer. Among the acidic conditions tested, any effects on lifespan are likely to be due to the composition of the buffer rather than a direct result of the lower pH. As evidence for this, we note that YPD buffered to pH 3.0, the most acidic condition tested, had no effect on lifespan.

In summary, we find no evidence that acidification of the culture medium, or pH changes in general, limit replicative lifespan in the BY4742 laboratory yeast strain under standard conditions. Buffer conditions that dramatically extend chronological lifespan of this strain do not similarly extend replicative lifespan. These data demonstrate that effects of acidification on aging in yeast are likely to be restricted to non-dividing cells.

Author contributions

MK and BMW conceived the study and designed the experiments. BMW, DC, HT, HD, NCS, JRN, JF, JL, BZ, JM, EO, TN, TS, AS and MT carried out the experiments. BMW performed the analyses of results. BMW and MK wrote the manuscript. All authors have critically reviewed and agreed to the content of the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by NIH Grant R01AG039390 to MK. BMW was supported by NIH Training Grant T32ES007032.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
References

1. Longo VD, Shadel GS, Kaeberlein M, et al.: Replicative and chronological aging in Saccharomyces cerevisiae. Cell Metab. 2012; 16(1): 18–31.

2. Steinkraus KA, Kaeberlein M, Kennedy BK: Replicative aging in yeast: the means to the end. Annu Rev Cell Dev Biol. 2008; 24(1): 29–54. Published Abstract | Publisher Full Text | Free Full Text

3. Fabrizio P, Longo VD: The chronological life span of Saccharomyces cerevisiae. Aging Cell. 2003; 2(2): 73–81. Published Abstract | Publisher Full Text

4. Steffen KK, Kennedy BK, Kaeberlein M: Measuring replicative life span in the budding yeast. J Cell Sci. 2009; (28). Published Abstract | Publisher Full Text | Free Full Text

5. Lin SJ, Defossez PA, Guarente L: Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science. 2000; 289(5487): 2126–2128. Published Abstract | Publisher Full Text

6. Schleit J, Johnson SC, Bennett CF, et al.: Molecular mechanisms underlying genotype-dependent responses to dietary restriction. Aging Cell. 2013. Published Abstract | Publisher Full Text

7. Powers RW III, Kaeberlein M, Caldwell SD, et al.: Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev. 2006; 20(2): 174–184. Published Abstract | Publisher Full Text | Free Full Text

8. Longo VD, Gralla EB, Valentine JS: Superoxide dismutase activity is essential for stationary phase survival in Saccharomyces cerevisiae. Mitochondrial production of toxic oxygen species in vivo. J Biol Chem. 1996; 271(21): 12275–12282. Published Abstract | Publisher Full Text

9. Ashrafil M, Sinclair D, Gordon JL, et al.: Passage through stationary phase advances replicative aging in Saccharomyces cerevisiae. Proc Natl Acad Sci USA. 1999; 96(16): 9100–9105. Published Abstract | Publisher Full Text | Free Full Text

10. MacLean M, Harris N, Piper PW: Chronological lifespan of stationary phase yeast cells: a model for investigating the factors that might influence the ageing of postmitotic tissues in higher organisms. Nast. 2001; 18(6): 499–509. Published Abstract | Publisher Full Text

11. Wei M, Fabrizio P, Madia F, et al.: TOR1/Sch9-regulated carbon source substitution is as effective as calorie restriction in life span extension. PLoS Genet. 2009; 5(5): e1000467. Published Abstract | Publisher Full Text | Free Full Text

12. Murakami C, Kaeberlein M: Quantifying yeast chronological life span by outgrowth of aged cells. J Cell Sci. 2009; (27). Published Abstract | Publisher Full Text | Free Full Text

13. Smith DL Jr, McClure JM, Matcic M, et al.: Calorie restriction extends the chronological lifespan of Saccharomyces cerevisiae independently of the Sir1/2 system. Aging Cell. 2007; 6(5): 649–662. Published Abstract | Publisher Full Text

14. Murakami C, Burtner CR, Kennedy BK, et al.: A method for high-throughput quantitative analysis of yeast chronological life span. J Gerontol A Biol Sci Med Sci. 2008; 63(2): 113–121. Published Abstract | Publisher Full Text

15. Burtner CR, Murakami C, Kennedy BK, et al.: A molecular mechanism of chronological aging in yeast. Cell Cycle. 2009; 8(8): 1256–1270. Published Abstract | Publisher Full Text | Free Full Text

16. Murakami C, Wolf V, Baxisty N, et al.: Composition and acidification of the culture medium influences chronological aging similarly in vineyard and laboratory yeast. PLoS One. 2011; 6(9): e24530. Published Abstract | Publisher Full Text | Free Full Text

17. Kaeberlein M, Powers RW III, Steffen KK, et al.: Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science. 2005; 310(5751): 1193–1196. Published Abstract | Publisher Full Text

18. Fabrizio P, Pozza F, Fletcher SD, et al.: Regulation of longevity and stress resistance by Sch9 in yeast. Science. 2001; 292(5515): 288–290. Published Abstract | Publisher Full Text

19. Fabrizio P, Fletcher SD, Minois N, et al.: Chronological aging-independent replicative life span regulation by Msn2/Msn4 and Sod2 in Saccharomyces cerevisiae. FEBS Lett. 2004; 571(1–3): 136–142. Published Abstract | Publisher Full Text

20. Delaney JR, Murakami C, Chou A, et al.: Dietary restriction and mitochondrial function link replicative and chronological aging in Saccharomyces cerevisiae. Exp Gerontol. 2013; 48(10): 1006–13. Published Abstract | Publisher Full Text | Free Full Text

21. Murakami C, Delaney JR, Chou A, et al.: pH neutralization protects against reduction in replicative lifespan following chronological aging in yeast. Cell Cycle. 2012; 11(16): 3087–3096. Published Abstract | Publisher Full Text | Free Full Text

22. Kaeberlein M, Kirkland KT, Fields S, et al.: Sir2-independent life span extension by calorie restriction in yeast. PLoS Biol. 2004; 2(9): e296. Published Abstract | Publisher Full Text | Free Full Text

23. Delaney JR, Chou A, Olsen B, et al.: End-of-life cell cycle arrest contributes to stochasticity of yeast replicative aging. FEMS Yeast Res. 2013; 13(3): 267–276. Published Abstract | Publisher Full Text

24. Smith ED, Kennedy BK, Kaeberlein M: Genome-wide identification of conserved longevity genes in yeast and worms. Mech Ageing Dev. 2007; 128(1): 106–111. Published Abstract | Publisher Full Text

25. Burtner CR, Murakami C, Olsen B, et al.: A genomic analysis of chronological longevity factors in budding yeast. Cell Cycle. 2011; 10(9): 1385–1396. Published Abstract | Publisher Full Text | Free Full Text

26. Leonleva OV, Blagoevkin MV: Yeast-like chronological senescence in mammalian cells: phenomenon, mechanism and pharmacological suppression. Aging (Albany, NY Online). 2011; 3(11): 1078–1091. Published Abstract | Publisher Full Text | Free Full Text

27. Kaeberlein M, Kennedy BK: A new chronological survival assay in mammalian cell culture. Cell Cycle. 2012; 11(2): 201–202. Published Abstract | Publisher Full Text | Free Full Text
Open Peer Review

Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 03 December 2013

https://doi.org/10.5256/f1000research.2624.r2625

© 2013 Dunn C. This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cory Dunn
Department of Molecular Biology and Genetics, Koç University, Istanbul, Turkey

In this work, Wasko et al. investigate whether altering the pH of rich medium can result in changes to the replicative lifespan of Saccharomyces cerevisiae strain BY4742. They find that replicative lifespan, except in the most extreme case of pH 9.0, is not affected by extracellular pH. Their results indicate that mutations and conditions increasing replicative lifespan are unlikely to do so through altering acidity/alkalinity of rich medium.

Recently, it was demonstrated by Hughes et al. Nature 492:261-265 that deletion of the vacuolar V₁ V₀-ATPase subunit Vma2p, which results in an increased vacuolar pH, causes a drastic shortening of replicative lifespan. Also, Hughes et al. found, by a qualitative microscopy assay, that over expression of VMA1 or VPH2 decreased vacuolar pH and that replicative lifespan was coincidentally increased.

Extracellular pH has been demonstrated to impinge on vacuolar pH, more prominently in the case of increased medium pH. This has been examined most comprehensively by Brett et al. PLoS ONE 6(3):e17619, but has also been demonstrated by others using genetic, fluorophore-based, and even 31P-NMR-based assays. If extracellular pH impinges upon vacuolar pH, and if replicative lifespan depends upon vacuolar pH, then why is replicative lifespan not changed upon altering extracellular pH? A discussion of this point is definitely warranted in a revised version of this manuscript.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 22 October 2013

https://doi.org/10.5256/f1000research.2624.r2096
Yeast cells have been successfully used for decades to identify conserved genetic and environmental factors that control cellular lifespan, which often underlies organismal lifespan. In yeast, the lifespan of dividing and non-dividing cells can be assessed via measurement of replicative and chronological lifespan, respectively. It was previously shown that chronological lifespan can be optimized through appropriate buffering of the cell culture media. In this brief F1000Research Research Article, Wasko et al., investigate whether replicative lifespan is also affected by environmental pH levels. The authors conduct a series of technically sound and straightforward experiments to address this question. Micromanipulation of BY4742 cells cultured under various pH conditions did not reveal any significant relationship between extracellular pH levels and replicative lifespan. The authors were quite careful in their interpretation of the findings and appropriately acknowledged limitations. For example, possible effects related to the different types of buffering agents used were noted. In addition, clear statements highlighting that the results of this study are obtained via reliance on a single yeast strain (BY4742) were included throughout the results and discussion sections. Moreover, statistical analyses were generally appropriate as presented. Overall, as suggested by the authors, the presented data suggest that the effects of acidification on aging in yeast are likely to be restricted to non-dividing cells. I only make the minor suggestion to consider adding the word “BY4742” before the word “yeast” in the title.

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
The benefits of publishing with F1000Research:

• Your article is published within days, with no editorial bias
• You can publish traditional articles, null/negative results, case reports, data notes and more
• The peer review process is transparent and collaborative
• Your article is indexed in PubMed after passing peer review
• Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com