***Saccharomyces cerevisiae* displays an increased growth rate and an extended replicative lifespan when grown under respiratory conditions in the presence of bacteria.**

Journal:	<i>Canadian Journal of Microbiology</i>
Manuscript ID	cjm-2017-0285.R1
Manuscript Type:	Note
Date Submitted by the Author:	15-Jun-2017
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Keyword:	Saccharomyces, symbiosis, volatile, lifespan, cross-kingdom

Saccharomyces cerevisiae displays an increased growth rate and an extended replicative lifespan when grown under respiratory conditions in the presence of bacteria.

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Abstract

Individual cells of the budding yeast *Saccharomyces cerevisiae* have a limited replicative potential, referred to as the replicative lifespan. We have found that both the growth rate and average replicative lifespan of *S. cerevisiae* cells are greatly increased in the presence of a variety of bacteria. The growth and lifespan effects are not observable when yeast are allowed to ferment glucose, but are only notable on solid media when yeast are forced to respire due to the lack of a fermentable carbon source. Growth near strains of *Escherichia coli* containing deletions of genes needed for the production of compounds used for quorum sensing or for the production of the siderophore enterobactin also still induced the lifespan extension in yeast. Furthermore, the bacterially induced increases in growth rate and lifespan occur even across gaps in the growth medium, indicating that the bacteria are influencing the yeast through the action of a volatile compound.

Keywords: *Saccharomyces*, symbiosis, volatile, lifespan, cross-kingdom

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Saccharomyces cerevisiae (yeast) divides asymmetrically. The asymmetrical budding allows one to track individual cells because the original or “mother” cell is larger than the “daughter” bud. Individual yeast cells have a finite replicative capacity referred to as the replicative lifespan (Longo et al. 2012; Smith et al. 2015). We have previously shown that respiring yeast cells have an extended replicative lifespan when grown on media supplemented with copper (Kirchman and Botta 2007). The copper is needed as a cofactor for an iron import protein, Fet3/Ftr1 (Stearman et al. 1996). The yeast supplied with supplemental copper are better able to import iron, resulting in an extended replicative lifespan (Botta et al. 2011).

Yeast also have the ability to acquire iron by hijacking siderophores, which are small molecules secreted to scavenge iron (Saha et al. 2013). To determine if yeast could get the supplemental iron they needed for an extended replicative lifespan from bacterial siderophores we grew yeast adjacent to *Escherichia coli*, which produces the siderophore enterobactin, which in some cases has been shown to promote growth of neighboring bacteria (D’Onofrio et al. 2010). Yeast grown next to *E. coli* (strain BW25113, *E. coli* genetic stock center, Yale University) were observed to divide more rapidly and displayed an extended replicative lifespan compared to yeast grown alone, however yeast grown next to *E. coli* unable to produce enterobactin (*entE* strain JW0586-1, *E. coli* genetic stock center, Yale University) (Baba et al. 2006) also displayed an increased growth rate and extended replicative lifespan that was not significantly different from the yeast grown near the wild type *E. coli* (Figure 1). Clearly *E. coli* cells were producing something other than enterobactin that was able to influence the yeast replicative lifespan. We performed the same assay next to *E. coli* strains unable to produce other known signaling molecules, AI-2, indole, or butyric acid (D’Onofrio et al. 2010) and all were able to induce the same extension of replicative lifespan in yeast (not shown).

We also found that the extension of replicative lifespan adjacent to *E. coli* required that the yeast were grown on media that forced them to respire (not shown). Given a fermentable carbon source, such as glucose, the standard carbon source used in yeast growth medium, *S. cerevisiae* will primarily ferment even in the presence of oxygen (Gancedo and Serrano 1989). In order to force respiration we used glycerol as the carbon source. We previously found that extension of replicative lifespan by copper supplementation also requires forcing yeast to respire (Kirchman and Botta 2007).

To determine if the extension of replicative lifespan and increased growth rate were specific to growth next to *E. coli* or a more general response, yeast were grown adjacent to several different species of bacteria. Most strains of bacteria assayed did elicit the extension of lifespan and increased growth rate in yeast, although not all did (Figure 2). In addition to *E. coli*, two other Gram negative bacteria, *Klebsiella pneumoniae* and *Proteus vulgaris*, elicited the increases, as did the Gram positive *Staphylococcus aureus*. One other Gram positive bacterium, *Micrococcus roseus* had no effect on the yeast lifespan or growth rate. Finally, *Pseudomonas aeruginosa* killed the adjacent yeast cells (not shown), a result that is not unexpected given the fact that *P. aeruginosa* is known to secrete compounds that can inhibit or kill *Candida albicans* (Morales et al. 2010; Morales et al. 2013).

Since most strains were secreting a substance or substances that influenced the lifespan of yeast we next sought to determine whether the substance(s) sole means of diffusion was through the media, or if volatile

components were involved. To accomplish this a gap, was cut in the growth medium between the bacteria and the yeast. Figure 3 shows that the lifespan of yeast across a gap in the media, while not as greatly increase as when directly adjacent, was still significantly increased compared to the control grown without bacteria. A corresponding increase in the growth rate of the yeast colonies was also obvious when yeast colonies were allowed to grow on plates either with or without bacteria, even across a gap in the agar (Figure 3). The decreased extension could be due to a reduced concentration of the compound required because of a loss of diffusion through the medium, or simply due to the fact that the gap in the agar created a greater distance between the yeast and bacteria. When we repeated the lifespan assay with yeast and bacteria separated by a greater distance the lifespan extension did decrease even without a gap in the agar (not shown). It is also possible that multiple compounds are inducing the extension and that one or more must diffuse through the medium while another (or others) are volatile.

Although not numerous, interactions between *S. cerevisiae* yeast and bacteria have previously been observed. While *P. aeruginosa* was lethal to *S. cerevisiae* in our hands, the growth of another *Pseudomonas* species, *Pseudomonas putida*, was enhanced by the presence of *S. cerevisiae* (Romano and Kolter 2004). *S. cerevisiae* exposed to N-acyl homoserine lactones, which are bacterial quorum sensing signal molecules, displayed changes in ethanol tolerance (Ren et al. 2016). Jarosz et al. (2014) described a mechanism by which bacteria induce the heritable induction of a yeast prion termed [GAR+], resulting in benefits for both the yeast and bacteria. We induced the [GAR+] state in strain BY4742, but [GAR+] did not result in the increased growth rate or lifespan observed when yeast were grown in the presence of bacteria (not shown). We also examined strains with deletions of the genes for chaperones Hsp104 or Hsp70, required for the induction of all known yeast prions (Brown and Lindquist 2009). Strains with each of these deletions retained the longer lifespans in the presence of bacteria (not shown), indicating the effect is not due to the induction of any known prion.

There are some very well know examples of cross-kingdom communication, such as the signaling that occurs between legumes and the nitrogen-fixing rhizobium bacteria that leads to root nodules (Damiani et al. 2016). Other examples between microbes occur in biofilms, such as the mutualistic interaction between *Streptococcus mutans* bacteria and the yeast *C. albicans*, which occurs in the human oral cavity (Sztajer et al. 2014) or the competitive interactions between *C. albicans* and *P. aeruginosa* bacteria that results in the production of virulence factors by *P. aeruginosa* (Trejo-Hernandez et al. 2014). Cross-kingdom communication by volatile compounds has been used in the lab to set up synthetic ecosystems (Weber et al. 2007) and logically seems like a mechanism that would be commonly found in nature, however there is little research available on naturally occurring examples.

Because of the availability of a variety of genetic tools and methods available with *S. cerevisiae*, it may be useful as a model system for exploring communication between bacteria and eukaryotes. Indeed, at least one study has used *S. cerevisiae* and the bacterium *Rhizobium etli*, to demonstrate how two species from different kingdoms with no expected history of interaction co-evolve in just a matter of weeks (Andrade-Domínguez et al. 2014).

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Figure 1. Yeast replicative lifespan is increased by growth adjacent to *E. coli*. Replicative lifespan assays were performed on YPG medium (2% peptone, 1% yeast extract, 3% glycerol, 2% agar) as previously described (Kirchman et al. 1999) except that yeast and bacterial colonies were grown overnight on plates prior to selecting cohorts. Cohorts of yeast cells were placed in a line approximately 50 μm from a patch of bacterial grown in the center of a 100 mm petri dish, or alone in the case of controls. Each cohort was assayed on its own plate. Cohort cells that did not produce at least 2 daughter buds were not included in the results. Lifespan analyses were repeated a minimum of 3 times and statistical results were obtained using a Mann-Whitney U test. A representative assay is shown, in which the replicative lifespan of yeast strain BY4742 was increased from a mean of 8.6 generations (■, n=24) when assayed alone to a mean of 32.4 (●, n=50) generations when grown next to *E. coli* ($p < 0.001$). Growth adjacent to an *entE*⁻ strain of *E. coli*, unable to produce the siderophore enterobactin, also resulted in an extension of mean replicative lifespan to 34.9 generations (○, n=50), which was significantly greater than yeast grown alone ($p < 0.001$) but did not differ significantly from yeast grown adjacent to the unmutated strain of *E. coli* ($p = 0.15$).

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Figure 2. Yeast replicative lifespan is increased by growth adjacent to many, but not all bacteria. The replicative lifespan of yeast strain BY4742 was significantly extended ($p<0.001$ in each case) from a mean of 7.2 generations (■, $n=25$) when grown alone, to 37.6 (*E. coli* □, $n=27$), 39.6 (*K. pneumoniae* ●, $n=27$), 31.7 (*P. vulgaris* ×, $n=30$), or 34.8 (*S. aureus* △, $n=25$) when grown adjacent to the indicated bacteria. Yeast replicative lifespan was not extended when cells were assayed adjacent to *M. roseus* (○), with a mean replicative lifespan of 6.8 ($p=0.17$, $n=25$).

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Figure 3. A volatile compound is involved in the increased yeast lifespan and growth rate. **A)** The replicative lifespan of yeast is increased from a mean of 8.9 generations (■, n=21) when grown alone, to 38.0 when grown adjacent to *K. pneumoniae* (●, n=21) ($p<0.001$), but also increases to a mean of 23.5 (○, n=22) ($p<0.001$) when the yeast and bacteria are separated by a gap cut in the agar. **B)** The growth rate of yeast colonies adjacent to bacteria is also observably increased when cells were grown to midlog phase in YPG liquid medium and 10-fold dilutions were spotted on YPG plates (2% agar) and grown for 2 days at 30°C either alone (top), adjacent to a streak of *K. pneumoniae* (middle), or on a plate with *K. pneumoniae* across a gap cut in the agar to physically separate the yeast and bacteria (bottom).

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