FUNGAL PHYSIOLOGY

Secrete to beat the heat

Cooperative behaviour enables populations of yeast cells to survive high temperatures.

Kevin R. Lauterjung, Neydis Moreno Morales and Megan N. McClean

Temperature affects growth rate, metabolism, morphology and reproduction in microorganisms. At optimal temperatures, growth and reproduction are efficient. But as temperatures increase, reactive oxygen species are produced and proteins denature, resulting in oxidative stress, growth cessation and death. A microorganism has upper and lower temperature limits for growth with an optimum at some point between these two extremes. *Saccharomyces cerevisiae* (budding yeast) grows optimally at ~35 °C and ceases growth above 40 °C. In this issue of *Nature Microbiology*, Laman Trip and Youk find that between habitable (~38 °C) and uninhabitable (~40 °C) temperatures, growth at 39 °C is dependent on population density[1].

Laman Trip and Youk experimentally mapped population-level yeast growth as a function of temperature and initial density (Fig. 1). The resulting phase diagram revealed that all yeast populations fail to survive in temperatures above 40.3 °C. As this tipping-point temperature is approached, dense yeast populations continue to grow, less dense populations vary between some growing and some not growing, and sparse populations do not grow at all. Transitions between growth and non-growth were shown to be sensitive to additional stressors. For example, yeast that constitutively overexpress a fluorescent protein, and therefore demand more cellular resources, had a lower tipping-point temperature. Laman Trip and Youk ruled out selection for heat-tolerant mutants or persister cells by demonstrating that sub-cultured survivors are not heat tolerant and that initial population decay rates are inconsistent with persister cells.

Laman Trip and Youk's results show that yeast are better equipped to survive high temperatures when surrounded by neighbouring cells. This finding is surprising, because the textbook view is that survival is influenced by each individual cell's response and fitness. Further, they find that the transition between habitable and uninhabitable temperature conditions is exquisitely sensitive to cell density. Such density-dependent behaviour has been observed in many ecological systems that demonstrate cooperativity[2]. Cooperation leads to an Allee effect, whereby at low densities, the population growth rate increases with population density[3]. This effect further leads to the catastrophic tipping point seen at 40.3 °C where a stable state of the system merges with the unstable state. Once past this tipping point, the only stable state available to the system is extinction. Similar behaviour is seen in many ecological systems[4], including microbial populations that demonstrate cooperativity[5,6]. One particularly relevant example, also in budding yeast, is the cooperative metabolism of sucrose by secreted invertase. Dilution of yeast populations growing on sucrose results in a strikingly similar pattern of density-dependent growth and a tipping point at high dilution rates[7].

Harmful reactive oxygen species are produced when cells are exposed to high temperatures. Therefore, Laman Trip and Youk hypothesized that glutathione, an important yeast antioxidant[8,9], might enable cooperative thermoprotection. They showed that glutathione accumulates in yeast cultures grown at high temperatures.

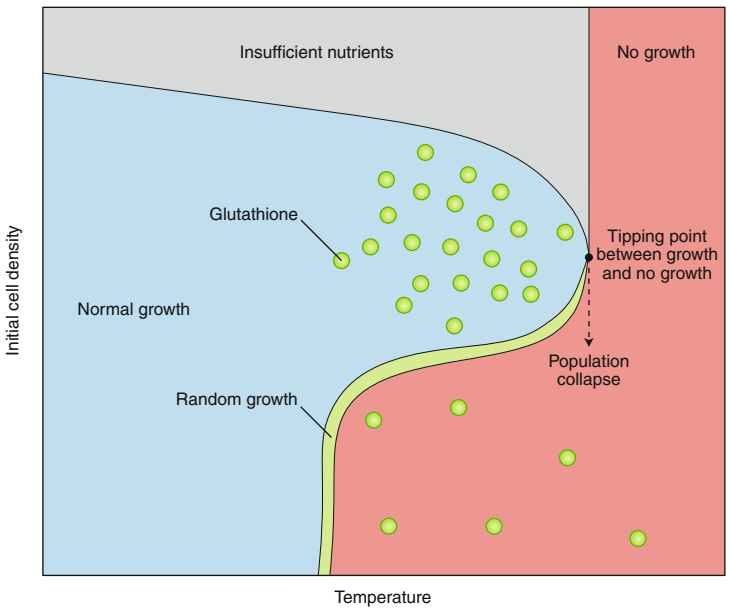

Fig. 1 | Cooperative secretion of glutathione extends the habitable temperature range for yeast. The growth of yeast populations was measured by Laman Trip and Youk as a function of initial cell density and temperature[1]. These conditions result in normal growth (blue region), random growth (populations that sometimes grow and sometimes do not; green region), no growth (red region) or no growth due to nutrient depletion (grey region). Past a catastrophic tipping point, where the stable fixed point (the boundary between normal growth and nutrient-limited regions) and the unstable fixed point (random growth region) collide, no growth is possible. Secretion of glutathione (green circles) by yeast leads to density-dependent growth at intermediate temperatures. Glutathione acts as an antioxidant, protecting yeast from cellular damage by reactive oxygen species. Too few cells results in insufficient amounts of glutathione produced and cells thus failing to divide (no growth/collapse). At higher cell densities, the cooperative production of glutathione protects yeast from heat damage and extends the habitable temperature range, resulting in growth.

Furthermore, spent media from these cultures or high concentrations of pure glutathione enabled growth when added to cultures that were otherwise too dilute to thrive at higher temperatures. Laman Trip and Youk generated and analysed mutations in genes known to be involved in glutathione transport and production, further demonstrating that the production and export of glutathione is essential to maintain growth at high temperatures, whereas importation is not. This suggests that the protection mechanism operates in the extracellular space. The authors built a stochastic mathematical model of yeast growth, in which the probability of replication is nonlinearly dependent on extracellular glutathione concentration. This model predicts population growth for different initial densities and temperatures, and fully recapitulates their experimental findings.

The authors' results suggest several research questions that could be investigated in the future. Although their data suggest that glutathione accumulation is necessary and sufficient for growth at high temperatures, it would be interesting to examine if other unidentified cooperative interactions also have a role. The mechanisms that allow some populations, but not others, to grow at higher temperatures could be examined in the context of variability in cell age, expression capacity and other factors. Thermotolerance in experimentally tractable yeast might be an interesting model system for examining early warning signals of population collapse[10], particularly in the context of global warming and climate change.

The study by Laman Trip and Youk updates the conventional view of how yeast combat heat stress. More broadly, it also challenges a view of microbial biology based on autonomous cells and instead indicates that we need to adopt a systems biology framework — on par with the dynamical systems of macroecology — for even the simplest and most well-understood behaviours. In the simple intraspecies system examined by Laman Trip and Youk, intercellular interactions give rise to emergent population-level phenotypes. In more complex communities, including multispecies communities or monospecies communities with metabolic specialization, more complicated interactions are possible, with correspondingly difficult-to-predict behaviours at the population level. Further experimental and theoretical research connecting measurable interactions with the ecology of microbial populations represents an important frontier in microbiology and carries many important implications for human and environmental health. ❐

Kevin R. Lauterjung[1], Neydis Moreno Morales[1] and Megan N. McClean[1,2] ✉

[1]Department of Biomedical Engineering, University of Wisconsin–Madison, Madison, WI, USA. [2]University of Wisconsin Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA.

✉*e-mail: mmcclean@wisc.edu*

Published online: 25 June 2020
https://doi.org/10.1038/s41564-020-0748-3

References

1. Laman Trip, D. S. & Youk, H. *Nat. Microbiol.* https://doi.org/10.1038/s41564-020-0704-2 (2020).
2. Clements, C. F. & Ozgul, A. *Ecol. Lett.* **21**, 905–919 (2018).
3. Allee, W. C., Emerson, A. E., Park, O., Park, T. & Schmidt, K. P. *Principles of Animal Ecology* (W. B. Saunders, 1949).
4. Scheffer, M., Carpenter, S., Foley, J. A., Folke, C. & Walker, B. *Nature* **413**, 591–596 (2001).
5. Artemova, T., Gerardin, Y., Dudley, C., Vega, N. M. & Gore, J. *Mol. Syst. Biol.* **11**, 822 (2015).
6. Veraart, A. J. et al. *Nature* **481**, 357–359 (2012).
7. Dai, L., Vorselen, D., Korolev, K. S. & Gore, J. *Science* **336**, 1175–1177 (2012).
8. Thorsen, M. et al. *Mol. Microbiol.* **84**, 1177–1188 (2012).
9. Grant, C. M. *Mol. Microbiol.* **39**, 533–541 (2001).
10. Scheffer, M. et al. *Nature* **461**, 53–59 (2009).

Competing interests

The authors declare no competing interests.