Escherichia coli chronological aging as a model to investigate common mortality patterns

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***Short Abstract* — Increasing mortality rate with age is a hallmark of aging. Demographic studies on human and model organisms have revealed a common mortality rate pattern consisting of an exponential increase and a slowdown later. The mechanisms governing these patterns remain unknown. Ww individually isolate E. coli cells in constant starvation conditions maintained by a microfluidic cell-array system, and showed that the bacterium exhibits mortality patterns similar to those of aging eukaryotes. Mutations perturbing the levels of cellular maintenance investments lead to drastic and additive changes in mortality patterns. Our study expands aging research to one of the simplest model organism.**

I. Background

AGING, often defined as the age-associated increase of mortality, is one of the most commonly seen features of all living forms. Ever since Benjamin Gompertz' law of human mortality, mortality curves have been taken as signatures of aging. Recent bio-demographic studies of various model organisms reveal common characteristics with human mortality patterns: a fast exponential increase with age after maturity and a deceleration at old ages [1]. Does the conservation of this pattern across such diverse group of organisms indicate the existence of a universal mechanism of aging [2]?

Recent studies demonstrated that aging is not restricted to eukaryotes and has been quantitatively observed in prokaryotes such as Escherichia coli [3]. However, despite the potential importance, accurate measurement of prokaryotic mortality patterns is still missing, due to bulk measurements which suffered from crucial effects on bacterial survivability caused by environmental variation and cross-feeding [4]. In this context we set to use microfluidic technology to quantitatively characterize the mortality pattern of E. coli, the most studied and understood biological model, at single cell resolution.

II. Results

We developed a lab-on-a-chip device to follow in real time chronological aging of E. coli. The system consists of a microfluidic device with micro-well arrays that physically immobilize a large population of individual E. coli with a one-cell-per-well manner. A constant flow is supplied to maintain homogeneous and controlled carbon-source starvation environment and to wash away rapidly any cellular content released from dead cells. The temporal viability profile of every bacterium is recorded using temperature-controlled time-lapse fluorescence microscopy.

A. Mortality rates of wildtype E.coli follows the pattern of those common observed for higher organisms.

Each experiments consists of about 10^4 cells initially. A repeatable pattern emerges: a first phase where mortality rates increase exponentially in the 80-100 hours, characterized by exponential exponent $\beta = 0.050 \pm 0.002 h^{-1}$, and a second 'plateau' phase with a near constant probability of death ($\beta = 0.000 \pm 0.002 h^{-1}$).

B. Mutations of the general response pathway (rpoS) have drastic yet additive effects on mortality rates

We find that mutations in the rpoS pathway which regulate balance between growth and maintenance lead to drastic changes in average lifespan (28% decrease in rpoS null mutant and 45% increase in rssB, which encode anti-sigma factor for RpoS.) However, the mortality curves of the two mutants share the same shape as the wild-type one, and unexpectedly, their chronological aging rates, i.e., the exponential factors of their mortality rates in the fast aging phase are not significantly different from that of wildtype, indicating that the mutations lead to multiplicative, delay-like, effects in E. coli mortality pattern.

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