Phenotypic heterogeneity of nutrient-starved *E. coli* cells

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***Short Abstract* — Bacteria in nature spend most of their life in a nutrient-deprived state. It is well known that when transferred to a nutrient agar plate, a large fraction of these nutrient-starved bacterial cells fails to form colonies. Currently, little is known about the physiological and metabolic states of these non-rejuvenating cells. Here, we characterized physiology, metabolism and gene expression of nutrient-starved *E. coli* cells with single-cell resolution. Our study reveals surprising phenotypic heterogeneity in nutrient-starved cells.**

***Keywords* — Quantitative single cell microbiology, bacterial physiology, starvation, growth**

I. Background

In nature, bacteria spend most of their lifetime in nutrient-depleted environments. When transferred to a nutrient agar plate, only a fraction of them forms colonies. A similar phenomenon was observed in laboratory experiments; when bacterial cells are deprived of nutrients, the number of cells that form colonies (when transferred to a nutrient agar plate) decreases by half within a couple of days (for reviews [1, 2]). Currently, little is known about physiological and metabolic states of these cells that fail to form colonies.

Here, employing time-lapse fluorescence microscopy and fluorescence markers, we characterized physiological and metabolic states of the non-rejuvenating cells.

II. Results

A. Correlating membrane integrity and nutrient-uptake ability to rejuvenation.

Cells that were previously growing exponentially were subjected to nutrient deprivation. At different times, we took an aliquot, treated the cells using propidium iodide (PI) and 2-NBDG (fluorescent glucose analog) and transferred them to a nutrient agar plate. PI and 2-NBDG report membrane integrity and nutrient-uptake ability, respectively. Then, using time-lapse fluorescence microscopy, we monitored the rejuvenation of cells with single-cell resolution. Cells that have intact membrane (unstained by PI) and nutrient uptake ability instantly rejuvenated. As more time elapses in starvation, the number of instantly-rejuvenating cells decreases; the decrease quantitatively agrees with decrease in the number of colony forming units (CFU).

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B. Phenotypic heterogeneity of non-rejuvenating cells.

Next, we characterized non-rejuvenating cells. We see that they consisted of (i) membrane disrupted cells (stained by PI), (ii) ghost cells (judged by poor phase contrast image), and, importantly, (iii) healthy looking (based on phase contrast image) cells with intact membrane and nutrient uptake ability. The fraction of the category (iii) cells was observed to be ~0% of the total population at the onset of the starvation, increased to ~5% within ~2 days, and remained about the same level afterwards (~for a week). When we monitor the category (iii) cells for an extended period of time, we see that they rejuvenate at much later times. Hence, they are dormant cells.

Further characterization reveals their lack of ability to produce proteins and, also, their lack of resilience during starvation.

III. Conclusion

Our finding reveals phenotypic heterogeneity of nutrient-deprived cells. Importantly, the high percentage of dormant subpopulation is of particular interest to microbial ecology and medicine.

References

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[2] Oliver JD, (2010) Recent findings on the viable but nonculturable state in pathogenic bacteria. *FEMS Microbiol Rev.* **34(4)**: 415-425.