Review of: "The evolution of E. coli is NOT driven by genetic variance but by thermodynamics."

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Potential competing interests: No potential competing interests to declare.

In the LTEE, each reference ancestor culture (12 cultures are considered) starts from the same level and attains a final steady level through a number of duplication steps, $b \approx 6.6$ in one day. However, after several generations, the observed one-day growth extent in a given culture "*I*" is larger than for the ancestor culture, "*J*": this increase is measured as "relative fitness", $W_{ij,}$ namely, the ratio b_i/b_j. W_{ij} first increases quite steeply, but, later on, it takes a much smaller pace, the overall shape reflecting a power law trend.

The one-day span seemed adequate to attain a steady population level, which is about 100 times larger than the starting level for either competing culture (Ara+, or ARA-) used to determine W_{ij}. This means that $W_{ij} > 1$, although not exceeding 1.7 after more than 20 thousands generations. For $W_{ij} = 1.5$ one should observe a steady population level of the culture "*I*" almost ten times larger than for culture "*J*". The raising incongruence is that, in such a case, the one-day growth extent would imply a population 1000 times larger than the starting level, which would exceed the expected limit allowed by the available substrate. This suggests that such larger-than-1 W_{ij} might come from a misevaluation (taking into account the wide error range related to plate count experiments), or, more intriguingly, that the culture system (medium + cells) of the ancestor "*j*" allows the attainment of a lower steady population density than the system of the culture "*I*".

Since no change concerns the medium, the reason for $W_{ij} > 1$ would mainly depend on the evolution of the cells that require a smaller amount of substrate to grow: they become "more efficient".

This behavior may reflect the maximum power principle [1, 2] of the non-equilibrium system (medium + cells), as suggested by the authors who call it "the principle of least action". Or, much better and specifically, it may reflect the increased **emergy** of the cells [3 -4], as the result of an improved capacity of surviving and duplicate, thanks to the energy supply from the substrate consumed by the preceding generations. In other words, the uptake of substrate allows not only capacity of duplication, but also improved use of the available free energy.

The consequence of such a behavior should concern not only the possibility to attain a larger population density with the same amount of available substrate, but also a quicker onset of the growth, namely, a shorter latency gap before the growth onset, a property that unfortunately remains untreated in the LTEE program. This may possibly depend on the misleading location of the latency gap [5]. The growth onset tail does not reflect a true lag phase, as the actual no-growth latency gap precedes the growth onset and is related to the growth extent [5].

Nonetheless, growth extent, latency gap and maximum specific growth rate are parameters of the whole system (medium + cells). The macroscopic evidence is the result of the concurrence of a number of microscopic and molecular events, which can differ from system to system, but anyway produce the collective behavior represented by the classical sigmoid profile of the growth curve that actually is the same for every microbial species [6].

The manuscript by Keith Baverstock and Arto Annila is worth publishing as it can stimulate further developments.

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