Variable Time-Delay to Commitment is the Major Source of Heterogeneity in Bacterial Spore Germination Kinetics

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***Short Abstract* — Some types of bacteria form spores during starvation and germinate into vegetative cells when conditions improve. The biochemical mechanism that initiates germination is not well understood. Here, we performed both experimental and theoretical studies on the germination kinetics of *Bacillus* spores, and clarified the relation between two of the major events at the earliest stage of germination.**

***Keywords* — nutrient germination, commitment to germination, dipicolinic acid release.**

Some species of gram positive bacteria can form spores during starvation. The spores are metabolically dormant and can remain so for a long time. However, when conditions for growth again become favorable, such as when nutrients reappear, spores can quickly germinate into vegetative cells [1]. One of the major events in this process is the release of spore's large depot of dipicolinic acid (DPA). The delay time between the introduction of nutrients and DPA release is widely variable within a spore population [2]. Despite decades of study, the factors giving rise to this heterogeneity are still unknown. Here, we addressed the origins of this heterogeneity by studying the relationship between DPA release and the first assessable event during germination — commitment.

Once commitment occurs nutrients may be removed but germination still proceeds [3]. This step of germination has been largely overlooked for many years until a recent study [4], which enabled the separate identification of commitment and DPA release under various conditions with various spore species and strains. However, the measurements were on populations (~10^7) of spores, while our goal is to clarify the relationship between the two events on single spore level. To take advantage of the new study and other recent studies, we developed a mathematical analysis which extracts a single spores' behavior from population measurements.

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Firstly, DPA release has been well characterized both on the population level [4] and on the single spore level [2]. Mathematically, the DPA release by a population of spores is the convolution of the distribution of the release time and a weight function characterizing the release by a single spore. Since both the population release and single spore release were measured experimentally, the release time distribution can be extracted.

Secondly, commitment was only measured on the population level [4]. However, we have shown that the commitment time distribution is simply proportional to the rate spores in a population commit to germination as a function of time.

Thirdly, the duration between commitment and DPA release may vary from spore to spore. The convolution of the distribution on this duration and the distribution of commitment time leads to the DPA release time distribution. We have shown that the average duration from commitment to DPA release is independent of the time of commitment and nutrient concentration, implying that the process after commitment is independent of the process triggering commitment.

The findings above highlight the importance of understanding commitment, which we feel is critical to identifying the mechanism of germination initiation. Currently, the only way to study commitment is on populations of spores as described above, but we are in the process of developing methods to extract the dependence of a single spore's commitment kinetics on different experimental conditions from population data, which will be an important step toward a more mechanistic understanding of how germination is initiated.

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