Genome **Biology**

RESEARCH HIGHLIGHT

Bet hedging in the underworld

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Abstract

Under starvation conditions, the soil bacterium *Sinorhizobium meliloti* divides into two types of daughter cell: one suited to short-term and the other to long-term starvation.

Soil-dwelling bacteria face the challenge of maintaining fitness in the face of frequent changes in nutrient availability. Many species have evolved mechanisms of food storage, which are likely to enhance survival during periods of starvation. The classic example is production of lipid-like poly-3-hydroxybutyrate (PHB) by bacteria in the family of Rhizobiaceae (collectively known as rhizobia). Rhizobia lead a dual life as both soil saprophytes, living off dead organic matter, and as symbionts of leguminous plants. As plant symbionts, they exist in specialized organs known as nodules, where they convert atmospheric nitrogen into ammonium for use by the plant. Within the nodule, rhizobial activities are powered by carbohydrates from the plant, with surplus being partitioned to the storage compound PHB [1]. Stored PHB - a reward from the symbiosis - can exceed 50% of the cellular dry weight of rhizobia and is used to support growth in the nutrient-limited bulk soil after rhizobia are released from senescing nodules.

Before reaching the next suitable host, rhizobia must decide how to use stored PHB. The critical but unknown factor is the length of time before a new host is encountered. How should the bacterium respond? One possibility is to use PHB as fast as possible, thus maximizing short-term growth rate - a strategy likely to be successful if a new host is encountered in the near future, but disastrous if there is a prolonged period before the next host. Alternatively, PHB could be used conservatively, thus increasing the likelihood of long-term survival - a strategy likely to be successful if new hosts are rarely encountered, but of limited utility if a new host is

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encountered in the near future. In the face of such uncertainty, it can pay to 'hedge one's evolutionary bets': to spread the risk of being maladapted in some future environment among variable offspring, each of which has a chance of being adapted to future conditions [2].

Low-PHB and high-PHB *S. meliloti* cells

In a recent paper, Ratcliff and Denison [3] suggest that rhizobial cells use a bet-hedging strategy to manage PHB storage. Under starvation conditions, cells divide to give rise to daughter cells of two contrasting phenotypes: high-PHB and low-PHB. Low-PHB cells are more competitive in saprophytic reproduction and are thus suited for short-term survival, whereas high-PHB cells survive longer without nutrients and are thus suited to withstand prolonged starvation. The authors [3] argue that co-existence of two phenotypes ensures greater long-term fitness, reduced variation across starvation events and high reproductive output over many rounds of plant-to-soil life cycles. (More specifically, they argue that it ensures greater long-term geometric mean fitness.)

The focus of study was *Sinorhizobium meliloti*, the microsymbiont of alfalfa (*Medicago sativa* L.), which accumulates PHB during plant symbiosis and also during stationary phase in laboratory medium (high- and low-PHB variants can be distinguished by flow cytometry). By way of support for the bet-hedging hypothesis, Ratcliff and Denison [3] showed that under starvation conditions, an initial population of high-PHB cells differentiates into two distinctive subpopulations of cells: one with high and the other with low PHB levels. They also showed that the phenotypic dimorphism is stable - even after more than 500 days of starvation. Microscopic analysis of dividing high-PHB cells showed that PHB granules are allocated asymmetrically. During cell division of rod-shaped bacteria, the ends of a cell are designated the old and new poles; PHB granules are preferentially retained in the old-pole cells of *S. meliloti*. Furthermore, the capacity to switch between high- and low-PHB cells was shown to be a heritable property of individual bacteria [3].

To see whether different phenotypes confer advantages under starvation conditions, the authors [3] compared the fitness of high-PHB cells relative to low-PHB cells at 14 days and then again at 528 days. No difference in cell viability was observed during short-term starvation

(14 days); however, after 528 days, the survival rate of high-PHB cells was five times greater than that of low-PHB cells. When grown in nutrient-rich medium, populations with a larger fraction of high-PHB cells had a significantly longer lag phase: as a consequence, populations with large numbers of high-PHB cells were less competitive than those with low numbers of high-PHB cells. Together, the fitness data suggest that high-PHB cells have a survival advantage under long-term starvation but a slower growth response to exogenous nutrients, whereas the low-PHB cells seem to be primed for rapid reproduction but are less capable of survival during long-term starvation [3].

Is the phenotype switching an adaptation to fluctuation selection?

Although the experiments reported by Ratcliff and Denison [3] are consistent with the hypothesis that switching between high- and low-PHB is a bet-hedging strategy, a key issue is whether this behavior is an adaptation, that is, whether it is an evolutionary response to fluctuating selection shaped by natural selection. Resolving this issue - as the authors mention - poses significant challenges [4]. Some insights, we suggest, are possible from the results of contemporary comparative and mechanistic studies.

Central to the PHB bet-hedging model is asymmetric cell division: unequal division on starvation generates daughter cells with high and low levels of PHB. *S. meliloti*, like the related bacterium *Caulobacter crescentus* (both members of the α-Proteobacteria), is known to undergo asymmetric cell division [5,6]. Observations from fluorescent microscopy show that *S. meliloti* cells typically divide to produce daughter cells that vary in length: old-pole cells are approximately 12% longer than new-pole cells [5]. This resembles the well-studied asymmetric division in *C. crescentus* in which stalked cells divide to give rise to swarmer cells. Molecular analysis of genes involved in asymmetric division, particularly those regulated by CtrA, which upregulates many genes involved in cell division, reveal a conserved mechanism of cell cycle control in *S. meliloti* and *C. crescentus* - and indeed in other members of the α-Proteobacteria [6]. For example, in *S. meliloti* the cell cycle regulator DivK is localized to one pole of the longer (old-pole) cells, but is not polarly localized in the shorter (new-pole) cells; similarly, DivK in *C. crescentus* is localized to one pole in stalked cells but shows no polar localization in swarmer cells [5].

These findings raise the possibility that, as in *C. crescentus*, asymmetric cell division in *S. meliloti* is a developmentally programmed event. As such, it calls into question the appropriateness of the bet-hedging framework. That a genotype produces entities with different morphologies and fitnesses is a necessary condition for bet hedging, but alone is not sufficient. A critical issue is evidence of a mean-variance fitness tradeoff (a reduction in temporal fitness variation at a cost of reduced mean fitness) [7]. A further requirement is some evidence of stochasticity in the underlying mechanism. Many organisms - including bacteria - show differentiation and complex development but would not be regarded as bet-hedging types. For example, the production of two genetically identical but functionally different progeny cells by *C. crescentus* - one flagellated but unable to reproduce and the other a stalked cell competent for replication - is an apparently successful adaptation for survival in environments in which resources are patchy [6]. Such a strategy would fit certain definitions of bet hedging, but reference to differentiation in *C. crescentus* as bet hedging makes little sense because the strategy is fixed by development.

A further and related issue concerns the capacity for a developmentally controlled phenotypic switch to be tuned to prevailing environmental conditions. Simple models [8] predict that, in organisms capable of bet hedging, the rate of switching between phenotypic states will evolve to match the rate of environmental change. Accordingly, for *S. meliloti*, it is reasonable to assume that isolates from environments that differ in the temporal and spatial patchiness of resources will show different rates of switching. For example, *S. meliloti* from resource-rich environments should evolve switching rates that are biased toward the production of low-PHB cells, whereas the opposite should hold for bacteria from resource-poor environments. However, if switching is tied to the cell cycle and subject to developmental control, then it is difficult to see how selection could lead to the evolution of bet hedging, let alone the tune switching rates to suit prevailing conditions.

Further work is needed to determine whether our alternative hypothesis - that switching in *S. meliloti* is developmentally regulated and akin to a *C. crescentus*-like life cycle - or the authors' hypothesis of selected bet hedging is the correct explanation for the observations of Ratcliff and Denison [3]. A great strength of the work stems from the authors' close and detailed attention to cellular variation. Many microbes show similar behaviors, but rarely is the significance of such phenotypic variation considered. Here, with focus on the ecological significance of variation at the cellular level and its evolutionary origins, the authors [3] open the door to new vistas in microbial ecology and evolution, with likely far-reaching consequences.

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