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Diversity of bet-hedging strategies in microbial communities—Recent cases and insights

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Abstract

Microbial communities are continuously exposed to unpredictable changes in their environment. To thrive in such dynamic habitats, microorganisms have developed the ability to readily switch phenotypes, resulting in a number of differently adapted subpopulations expressing various traits. In evolutionary biology, a particular case of phenotypic heterogeneity that evolved in an unpredictably changing environment has been defined as bet-hedging. Bet-hedging is a risk-spreading strategy where isogenic populations stochastically (randomly) diversify their phenotypes, often resulting in maladapted individuals that suffer lower reproductive success. This fitness trade-off in a specific environment may have a selective advantage upon the sudden environmental shift. Thus, a bet-hedging strategy allows populations to persist in very dynamic habitats, but with a particular fitness cost. In recent years, numerous examples of phenotypic heterogeneity in different microorganisms have been observed, some suggesting bet-hedging. Here, we highlight the latest reports concerning bet-hedging phenomena in various microorganisms to show how versatile this strategy is within the microbial realms.

This article is categorized under:

Infectious Diseases > Molecular and Cellular Physiology

KEYWORDS

adaptation, bet-hedging, evolutionary strategy, persisters, phenotypic heterogeneity

1 | INTRODUCTION

In many natural environments, microbial populations are constantly exposed to fluctuations of biotic and abiotic factors. For instance, soil-inhabiting microorganisms like *Bacillus subtilis* sense frequent changes of osmolarity caused by interchanging rain and drought periods and accordingly regulate the transport and biosynthesis of osmoprotectants (i.e., proline and glycine betaine) to avoid further cell rupture or desiccation (Bremer & Krämer, 2019). Therefore, microorganisms must evolve various adaptation strategies to sense and process environmental information readily and avoid extinction.

In a direct response to challenging environmental conditions, microorganisms exploit various gene regulatory networks, such as operons and regulons, to modulate their phenotype and/or behavior (Benson & Haldenwang, 1992;

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Crombach & Hogeweg, 2008; Jacob & Monod, 1961; Krell et al., 2010; Siebring et al., 2012). The capacity to adapt by reversibly switching between different phenotypic states, analogously to ON/OFF switches, is known as phenotypic switching (Henderson et al., 1999; van der Woude & Bäumlner, 2004). However, phenotypic switching usually occurs only in a fraction of the population due to the presence of intra- and extracellular noise and the topology of regulatory networks involved in the sensing and processing of the environmental signals (i.e., bi- or multi-stable networks; Elowitz et al., 2002; Ozbudak et al., 2002; Paulsson, 2004; Pedraza & van Oudenaarden, 2005; Veening, Smits, et al., 2008). As a result, a nongenetic differentiation within an isogenic population gives rise to several phenotypically distinct subpopulations. This phenomenon is known as phenotypic heterogeneity, and some of its examples include lactose utilization in *Escherichia coli* (van Hoek & Hogeweg, 2007), cellular differentiation in *B. subtilis* (Kearns & Losick, 2005; Smits et al., 2005; Veening, Igoshin, et al., 2008; Yüksel et al., 2016), flagellin phase variation in *Salmonella enterica* (Bonifield & Hughes, 2003), and the development of stress-resistant yeast (Bishop et al., 2007).

Phenotypic heterogeneity may arise from a responsive event to specific environmental cues (responsive switching), but it can also be the result of random changes in gene expression that are independent of the varying environmental conditions (stochastic switching) (Elowitz et al., 2002; Kussell, 2005; Levine et al., 2013). These strategies have different advantages and disadvantages for populations exposed to certain fluctuating environmental conditions (Figure 1). Since responsive switching strongly depends on the maintenance and the activation of stress-specific sensory circuits, it causes an adaptive lag that can be critical for survival when the environment fluctuates (Acar et al., 2008; Kaern et al., 2005). In unpredictable environments, stochastic switching can be advantageous over responsive switching by generating a variety of maladapted phenotypes (i.e., phenotypes with reduced fitness), which overall increase the long-term fitness of the population (Ackermann, 2015; Kussell, 2005; Kussell et al., 2005). This particular form of phenotypic heterogeneity, in which the individuals stochastically express maladapted phenotypes, is known as “bet-hedging.”

In evolutionary biology, bet-hedging has been described as a risk-spreading strategy displayed by isogenic populations that explicitly evolved in fluctuating environments (Gillespie, 1974; Kussell, 2005; Philippi & Seger, 1989). Under such conditions, clonal populations stochastically generate phenotypes with different fitness-related traits, resulting in individuals suffering lower reproductive success. Because the environmental changes favor different phenotypes at different times, the presence of randomly fit individuals may have a selective advantage when the sudden environmental shifts occur. Populations that employ bet-hedging minimize the temporal fitness variance of surviving offspring and maximize the long-term geometric mean fitness across generations (de Jong et al., 2011; Grimbergen

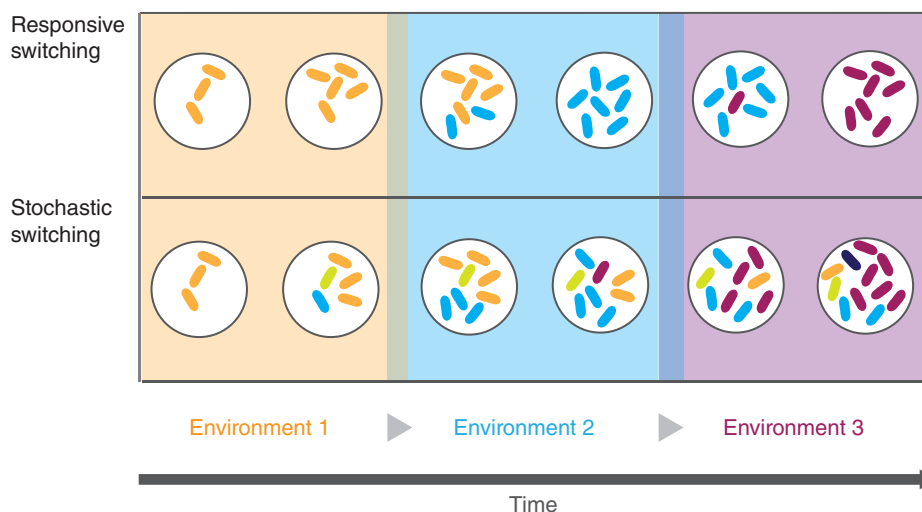


FIGURE 1 Responsive versus stochastic switching (adapted from Kussell et al., 2005). Isogenic cell populations adapt to changing conditions by switching their phenotype, either responsively (upper panel) or stochastically (lower panel). A schematic representation of the switching strategies is shown, in which the color of the fittest individuals matches the color of the environment. In responsive switching, cells change their phenotype when sensing an environmental change to maximize temporal fitness. The population survives if the majority of the individuals successfully commit to the switch. However, when the environment changes in a stochastic manner, the stochastic switching strategy becomes critical for adaptation. Populations that randomly employ stochastic switching, express a number of maladapted phenotypes of reduced fitness that may suit another environment in the future

et al., 2015; Kussell et al., 2005; Seger, 1987; Starrfelt & Kokko, 2012). Importantly, this temporal trade-off between fitness mean and variance does not occur in any other adaptation strategies.

One of the most prominent and well-studied cases of how microbes use a bet-hedging strategy is the formation of persister cells in different bacteria, including *E. coli*, *S. meliloti*, or, in more recently reported studies, *Staphylococcus aureus* and *Caulobacter crescentus* (Balaban et al., 2004; Huang et al., 2020; Keren et al., 2004; Kussell et al., 2005; Zalis et al., 2019). In this mechanism, under antibiotic stress conditions, a part of the initially isogenic population stochastically and reversibly enters into dormancy and becomes resistant to a killing dose of the antibiotics (Figure 2). Furthermore, once the antibiotic pressure is relieved, the dormant cells can regrow and repopulate the environment. In this regard, the presence of persister cells becomes highly relevant in common antibiotic treatments (Fisher et al., 2017; Lewis, 2007, 2010).

Theoretical studies have shown that the evolution and success of bet-hedging strategies depend on many factors, including the environment's reliability, that is, the frequency and magnitude of environmental changes (Gaál et al., 2010; Kussell, 2005); the ability of the population to respond to changes by mutations and rare phenotype selection (King & Masel, 2007; Wolf et al., 2005); the presence of a suitable environmental cue at the time (King & Masel, 2007); and the co-occurrence of other evolutionary strategies (King & Masel, 2007). Considering all these ecological factors, it is very challenging to provide empirical evidence for true bet-hedging strategies for several reasons. First, bet-hedging traits evolve in isogenic populations, and since the microbial genomes are subjected to natural genetic modifications or acquired mutations, the genetic composition of the studied population should be considered in the experimental setup. Second, because adaptive changes usually arise after long periods, and the fitness gains must be quantified across several generations, it is necessary to perform long-term evolutionary experiments under fluctuating growth conditions.

Consequently, authors of recent reports have discussed six categories of experimental evidence for bet-hedging strategies (de Jong et al., 2011; Grimbergen et al., 2015; Simons, 2011). Until now, only a few studies have experimentally

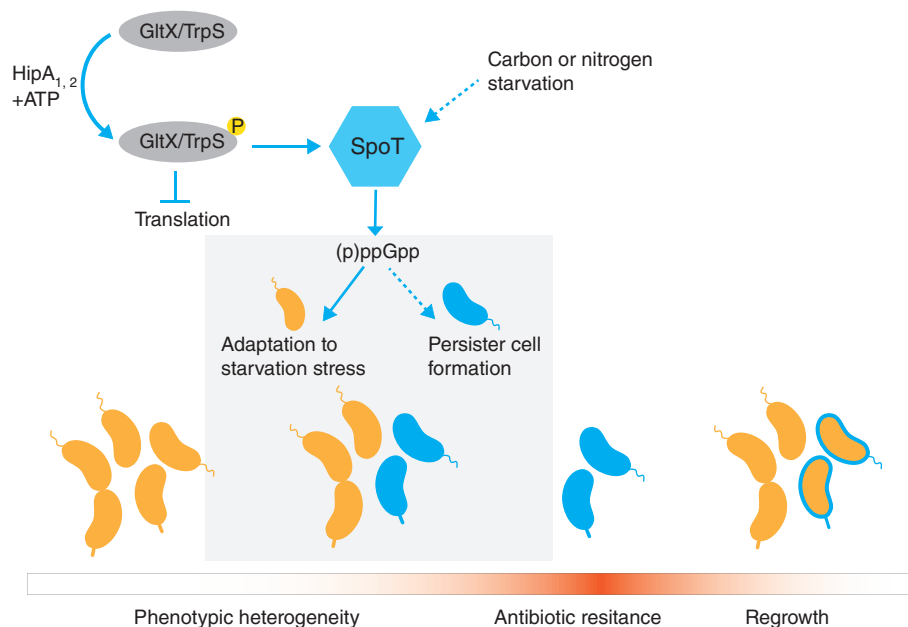


FIGURE 2 Schematic representation of persister cell formation in *Caulobacter crescentus*. It has been proposed that antibiotic persistence in *C. crescentus* is promoted by HipA1 and HipA2 toxins, which are serine/threonine kinases that phosphorylate the aminoacyl-tRNA synthetases GltX and TrpS, preventing synthesis of charged tRNAs (Huang et al., 2020). Phosphorylation of GltX/TrpS leads to translation arrest and activation of the amino acid starvation-signaling pathway (SpoT). Activation of SpoT is also stochastically triggered by carbon or nitrogen starvation (indicated by the dashed arrow). Elevated levels of (p)ppGpp, a stringent response alarmone, contribute to translational arrest. Activation of SpoT and further transcriptional changes in the isogenic population of *C. crescentus* allow most cells to adapt to the starvation conditions, whereas only a fraction of the population becomes dormant (phenotypic heterogeneity). Dormant cells (blue cells) are not metabolically active and can survive high doses of antibiotics. The persister state is reversible; therefore, when the optimal conditions arrive, dormant cells can repopulate the environment (orange cells). The gradient red-colored bar indicates starvation stress

demonstrated stochastic phenotypic switching in dynamic environments, including the elegant work of Beaumont et al. (2009), which provided the evidence for de novo evolution of bet-hedging traits in *Pseudomonas fluorescens* under frequently fluctuating conditions (Beaumont et al., 2009). Here, selection of phenotypes was achieved by repeatedly imposing the exclusion rule and a population bottleneck. Applying both at the point of environmental shift enabled to maintain diversity in the population by excluding the most common phenotype (the exclusion rule) and to select for a random variant among the surviving cells to minimize the cost of bet-hedging (a bottleneck). Interestingly, the authors showed that in two out of 12 experimental replicates, surviving genotypes persisted due to stochastic phenotype switching. Furthermore, in one of the switching genotypes, they identified a mutation in the *carB* gene, encoding for a subunit of carbamoylphosphate synthase involved in pyrimidine and arginine biosynthesis. It is speculated that this mutation caused significant changes in the central metabolism of the evolved population that further translated to molecular noise and promoted stochastic phenotype switching.

This experimental example was later revisited by Libby and Rainey (2011). Here, the authors used mathematical models and simulations to investigate the benefits of stochastic switching in populations of *P. fluorescens* subjected to the exclusion rule and population bottleneck (Libby & Rainey, 2011). Importantly, they observed that switching populations could invade and even replace the nonswitchers despite the high fitness costs and the frequency with which the switching occurred. Besides, the simulations showed that the results are robust to alterations in switching rate, the fidelity of exclusion, bottleneck size, duration of environmental state, and growth rate.

Both studies demonstrated that the exclusion rules and bottlenecks can shape the adaptation of populations responding to fluctuating conditions and that experimental studies, reinforced with theoretical models, can be a superior strategy in proving the evolution of bet-hedging. Nonetheless, much more remains to be discovered, including the mechanisms of stochastic switching, which is the most challenging to follow.

This mini-review discusses the most recent empirical studies where authors claim the role of bet-hedging in observed phenotypic heterogeneity. Specifically, we focus on describing microbial systems that display a bet-hedging strategy, but without stressing the six categories of experimental evidence proposed by de Jong et al., 2011, Grimbergen et al., 2015 and Simons, 2011. In Figure 3, we compiled all the examples discussed that fall into diverse microbial lifestyle areas, including signaling, resource use and dormancy.

2 | NOISE IN MICROBIAL CONVERSATIONS: BET-HEDGING IN QUORUM-SENSING RESPONSES AND CELL FATE DETERMINATION

It has been long recognized that cells within a microbial community can sense changes in the cell population density and adjust their gene expression accordingly (Nealson et al., 1970). This cell-to-cell communication mechanism is known as “quorum sensing” (QS), and is based on the production and sensing of small diffusible molecules known as autoinducers (AIs). AIs trigger cooperative behaviors and the production of “public goods” (Fuqua et al., 1994, 2001; Ng & Bassler, 2009; Williams, 2007). In addition, their chemical nature differs depending on the producing organism. For instance, Gram-negative bacteria release and sense homoserine lactones (Eberhard et al., 1981), whereas Gram-positive bacteria produce and sense autoinducing peptides (Kleerebezem et al., 1997; Magnuson et al., 1994). The QS signaling is AI concentration-dependent, reflecting the number of cells in the population (“quorum”). Accordingly, when the concentration of AIs reaches a threshold, QS-signaling promotes collective phenotype switching, which is beneficial for the entire population.

Processes reported to be controlled by QS include competence initiation (Håvarstein et al., 1996; Magnuson et al., 1994; Salvadori et al., 2019), endospore formation (Lazazzera et al., 1997; Li et al., 2011; López & Kolter, 2010), bioluminescence (Eberhard et al., 1981; Fuqua et al., 1994; Gray & Garey, 2001), secretion of virulence factors (Ji et al., 1995; Rutherford & Bassler, 2012), bacteriocin production (Brurberg et al., 1997; Kleerebezem, 2004; Kuipers et al., 1998; Shanker & Federle, 2017), biofilm formation (Brindhadevi et al., 2020; Hammer & Bassler, 2003; Kievit et al., 2001; Yarwood et al., 2004), and even changes in the bacteriophage lifecycle (i.e., lysogenic vs. lytic; Harms & Diard, 2019; Høyland-Krogsho et al., 2013; Qin et al., 2017). These experimental works describe QS-signaling in bulk populations in which the cells at a high density collectively contribute to the common good. Nevertheless, phenotypic heterogeneity is highly present in QS systems, mainly because QS can exploit bistable switches (Bauer et al., 2017). Some examples of phenotypic heterogeneity in QS include the LuxR/LuxI bioluminescence system of *Vibrio fischeri* and *Vibrio harveyi* (*campbellii*) (Anetzberger et al., 2009; Weber & Buceta, 2013), the presence of “social cheaters” in *Pseudomonas aeruginosa* (Sandoz et al., 2007), and the escape of

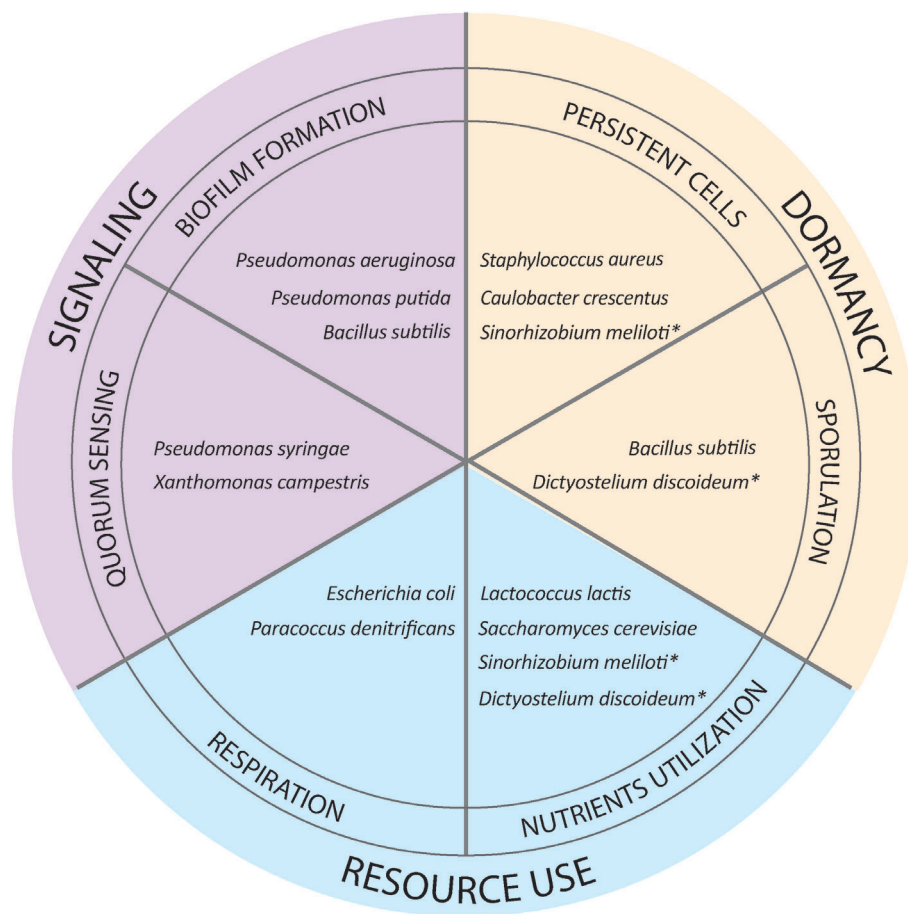


FIGURE 3 Overview of recent studies on phenotypic heterogeneity and possible employment of bet-hedging strategies in various microorganisms. In this work, we highlight some recent studies regarding bet-hedging traits that fall into several categories of microbial lifestyle, including signaling (purple), dormancy (yellow), and resource use (blue). In some cases, the same population manifests different bet-hedging strategies because of direct or indirect relationships between traits. With an asterisk, we marked examples of studies where a population was shown to employ several bet-hedging strategies, for example, nutrient utilization is directly involved in spore or persisters formation

individual cells from *Pseudomonas putida* biofilms (Cárcamo-Oyarce et al., 2015). Other examples of single-cell studies on QS and phenotypic heterogeneity were collected and neatly presented in the recent reviews by Grote et al. (2015), Mukherjee and Bassler (2019), and Bettenworth et al. (2019).

Interestingly, the occurrence of phenotypic heterogeneity in several cases of QS systems has been suggested to represent a potential bet-hedging strategy. Pradhan and Chatterjee (2014) used *Pseudomonas syringae* and *Xanthomonas campestris* to study bet-hedging in QS response to AIs (Pradhan & Chatterjee, 2014). To follow the phenotypic variation in response to AIs, they used chromosomally encoded green fluorescent protein reporter fusions, responsive to *N*-acyl-homoserine lactone and diffusible signal factor (cis-11-methyl-2-dodecenoic acid), respectively. In both experimental conditions, the authors show the coexistence of two subpopulations: QS-responsive and nonresponsive cells (bright and dark accordingly), at high cell-density in the presence of external QS signals. Moreover, this phenotypic heterogeneity has been identified as a nonheritable and reversible stochastic event, similar to persister cell formation when antibiotic pressure is removed. Induced and uninduced cells could further differentiate to QS-responsive or nonresponsive subpopulations, independently of their progeny. Since QS signaling is involved in coordinating multiple social behaviors, like motility or biofilm formation, the authors propose that the production of QS nonresponsive cells would be advantageous under rapidly changing conditions. However, despite the analogy of persister cells' formation, we suggest that in future studies long-term experiments and fitness estimations are necessary to prove that these mechanisms indeed evolved as a bet-hedging strategy.

2.1 | Spore formation in *B. subtilis*

In *B. subtilis*, the production of highly resistant endospores in response to nutritional stress is a prevalent example of an evolved bet-hedging strategy (Siebring et al., 2014; Veening et al., 2005). Spore formation is regulated by a multicomponent phosphorelay, in which the final threshold concentration of phosphorylated Spo0A, the key sporulation transcription factor, triggers spore-related gene expression (Fujita et al., 2005; Perego & Hoch, 2014). The Spo0A phosphorylation and thus sporulation initiation depends on several other signaling systems, including RapA-PhrA, a QS duo that responds to starvation stress (Perego & Hoch, 2014), and the two-component signal transduction system ComA-ComP, which indicates the cell density of the population and activates the RapA phosphatase (Lazazzera et al., 1997, 1999; Mueller et al., 1992). Remarkably, noise in the expression of the RapA-PhrA system has been reported as a source of heterogeneity in sporulation in *B. subtilis* (Veening et al., 2005). Previous studies demonstrated that cells with a low transcription of *rapA* sporulate early, whereas cells that strongly upregulate *rapA* delay their entry to sporulation (Bischofs et al., 2009). Later, Mutlu et al. (2018) showed that RapA-PhrA determines not only the sporulation timing, but also impacts the spore yield and the spore revival frequency (Mutlu et al., 2018). This phenotypic spore memory that differentiates spores into high and low quality is proposed to be a bet-hedging strategy to overcome the uncertainty of the environment, where the concentration of nutrients fluctuate. Early spores germinate more efficiently in an environment with low nutrient concentration than late spores, resulting in a fitness benefit under starvation periods. This hypothesis has been further validated theoretically and experimentally by Mutlu et al. (2020). In this work, the authors studied spore revival of *B. subtilis* strains isolated from gut and soil upon nutrient supply to determine the fitness advantage of different sporulation strategies, emphasizing spore quantity versus quality, and their evolutionary course (Mutlu et al., 2020).

2.2 | Sporulation and biofilm formation interplay

Cell fate determination in *B. subtilis* largely depends on bistable regulatory switches due to their noisy nature, hence a broad range of different phenotypes can be observed within an isogenic population. It has been long recognized that the *B. subtilis* biofilms are multicellular communities comprising motile, matrix-producing, and sporulating cells (Vlamakis et al., 2008). Cell differentiation is known to be tightly regulated by the levels of the phosphorylated Spo0A and depending on the intracellular concentration of Spo0A~P, a cell commits either to sporulation (high levels of Spo0A~P) or biofilm matrix formation (intermediate levels of Spo0A~P). In the case of biofilm development, the presence of an adequate concentration of Spo0A~P is essential for the production of SinI. SinI is a direct antagonist of transcription repressor SinR, which allows for transcription of *epsA* and *tasA* operons required for polysaccharide synthesis and production of amyloid fibers, by titrating SinR levels. Moreover, the levels of Spo0A~P and cell fate determination can be indirectly affected by other regulatory systems like DegU (responsible for floating biofilm formation) and the QS systems.

In *B. subtilis*, the ComX signaling peptide, a component of the ComA-ComP QS system, has been shown to positively regulate transcription of the *epsA-O* operon and polysaccharide synthesis by increasing surfactin production. Subsequently, an elevated surfactin concentration activates KinC, a histidine kinase which increases Spo0A~P levels, promoting biofilm formation. In the recent work of Spacapan et al. (2019), the role of QS in cellular development was investigated. The authors examined the role of ComX crosstalk between sporulation and biofilm formation and showed that the ComA-ComP QS system in floating biofilms reduces the growth rate and increases early stochastic sporulation, which suggests a potential bet-hedging strategy (Spacapan et al., 2019). The authors used a QS mutant named $\Delta comQ$, which does not produce active signal peptide ComX, and subsequently followed cell differentiation in floating biofilms to prove their hypothesis. By measuring the colony forming units and the number of spores in floating biofilms, the authors showed that floating biofilm of a wild type had a significantly higher amount of spores than the $\Delta comQ$ mutant. Moreover, the analysis of activation of the early sporulation promoter *PspoIIQ* confirmed that the $\Delta comQ$ mutation delays entry into sporulation. Interestingly, when the culture was supplemented with external ComX peptide, the promoter's activity resembled the wild type.

Taken all together, ComX was shown to act as a switch that regulates population growth and promotes early sporulation. In this regard, it has been proposed that early sporulation might benefit *B. subtilis* survival in fluctuating environments where nutrients are often depleted. This benefit is in line with those observed in the spore quantity versus quality trade-off described in the previous section. Therefore, it is tempting to speculate that this long-term fitness benefit is the result of a bet-hedging strategy.

3 | BET-HEDGING IN RESOURCES UPTAKE AND UTILIZATION

3.1 | Bet-hedging in respiratory mechanisms: a safety valve under anoxic spells

Microorganisms inhabiting microenvironments, where oxygen availability is limited (i.e., soil, sediments, biofilms, or marine environments), have developed respiratory mechanisms to utilize alternative oxidizing agents and ultimately generate energy to grow. Prominent examples are denitrifying bacteria, which use nitrogen oxyanions and oxides as electron acceptors during hypoxic/anoxic conditions (Shapleigh, 2006). Denitrifiers reduce nitrate to N_2 via nitrite, NO, and N_2O stepwise to avoid the entrapment in anoxia for long periods. For each step of reduction: NAR ($NO_3^- \rightarrow NO_2^-$), NIR ($NO_2^- \rightarrow NO$), NOR ($2NO \rightarrow N_2O$), and NOS ($N_2O \rightarrow N_2$), they express a set of reductases, which are encoded by *nar*, *nir*, *nor*, and *nos* gene clusters, respectively. The functionality and cooperation of all reductases prevent the accumulation of toxic intermediates like nitric oxide and conserve energy under frequent fluctuation in oxygen levels (Bergaust et al., 2008; Sullivan et al., 2013).

In the model denitrifier microorganism *Paracoccus denitrificans*, it has been observed that all cells in an isogenic population synthesize NOS, while only part of the population synthesizes NIR (Lycus et al., 2018). Lycus et al. (2018) described this phenotypic variation based on the NIR system's stochastic initiation, which eventually becomes autocatalytic via NO production. It is suggested that by employing stochastic initiation of NIR, *P. denitrificans* can hedge its bets and generate different cell variants under imminent anoxia. In this work, the authors propose that the bet-hedging strategy in NIR initiation is beneficial for conserving energy when the oxygen levels suddenly increase. In this scenario, the cell does not spend its energy synthesizing a denitrifying proteome (Olaya-Abril et al., 2018); instead, energy is invested in aerobic respiration (a long-term fitness benefit). However, the NIR expression, in sudden anoxia might cause a severe fitness loss (decreased growth rates caused by graduate NIR expression). Moreover, the authors provide evidence that NIR is localized and stored at the poles of nongrowing cells, ready to use in the case of a sudden switch to anaerobic respiration. Additionally, they discuss the effect of temperature on synthesizing one of the NIR components, NirS. Because the probability of NirS synthesis and expression of the complete denitrification proteome increased with increasing temperatures the authors suggest that the bet-hedging strategy might have evolved at low temperatures.

This elegant study highlights not only the evolution of a bet-hedging population. One can speculate that it also suggests a more profound complexity with a mix of survival strategies, where evolved bet-hedging crosses with the division of labor. The formation of different cell variants in a population of *P. denitrificans* is thought to contribute to survival via producing common goods (N_2O), cross-feeding interactions, and scavenging by neighboring cells. In this scenario, the division of labor might have evolved from the previous maladaptation that became beneficial on a generation-wide scale.

It has been recently suggested that not only denitrifying bacteria play the odds in environments when oxygen is scarce. Carey and Goulian (2019) proposed that the aerobically growing population of *E. coli* hedge its bets to prepare for the future anoxic spells by noisy expression of the *torCAD* operon (Carey & Goulian, 2019). The signaling two-component system TorT/TorS/TorR regulates the *torCAD* operon and allows *E. coli* cells to sense and utilize trimethylamine oxide (TMAO) as a final electron acceptor instead of oxygen. Previous studies on *torCAD* have shown that TorT/TorS/TorR complex is expressed as well under aerobic conditions, however with a high cell-to-cell variance of the mean expression values (Ansaldi et al., 2007; Carey & Goulian, 2019; Roggiani & Goulian, 2015). Consequently, the noisy expression generates different phenotypic variants resulting in maladapted cells' subpopulations with lower fitness under aerobic conditions. It has been shown that only cells that highly express the *torCAD* operon upon sudden anoxia continued to grow in anaerobic conditions, suggesting the bet-hedging strategy (Carey et al., 2018).

3.2 | Bet-hedging in nutrient utilization

Microorganisms require several nutrients to thrive in the niche they occupy, and previous studies illustrate how some hedge their bets in response to specific environmental conditions (Solopova et al., 2014; van Boxtel et al., 2017; Veening, Smits, et al., 2008). For instance, when a population of the amoeba *Dictyostelium discoideum* encounters nutrient depletion, two subpopulations are distinguished: aggregators (spores) and nonaggregators (vegetative cells; Wonhee & Gomer, 2011). An elegant theoretical work on the study of each subpopulation under short and long starvation conditions provided evidence of the bet-hedging strategy underlying this case of phenotypic heterogeneity (Martínez-García & Tarnita, 2017). This work demonstrates that more spores are formed in the long starvation condition, whereas

vegetative cells are predominant in the short starvation condition. Therefore, although one phenotype might do better at specific requirements, bet-hedging ensures the population's survival.

Similarly, a bet-hedging mechanism in response to starvation in the bacterium *S. meliloti* has been described (Ratcliff & Denison, 2010, 2011; Zhang & Rainey, 2010). In this work, under starvation conditions, two different cell types emerge after cell division, cells carrying either low or high concentrations of polyhydroxybutyrate (PHB; Ratcliff et al., 2008). The cell having high PHB concentrations can survive for long-term starvation periods. Besides, the authors of this work correlate this bet-hedging strategy with bacterial persistence because the high-PHB-containing cells show higher tolerance to the antibiotic ampicillin (Ratcliff & Denison, 2011). Importantly, this study provides evidence of the origins of the state of persistence triggered by a nutrient-stress and bet-hedging mechanism. In contrast to high-PHB-containing cells, the low-PHB cells are metabolically active, that is, they are nonpersistent cells, and thus this subpopulation of cells can utilize the nutrients that are available in the environment.

3.3 | Bet-hedging for amino acids utilization

Few studies have investigated bet-hedging strategies on amino acid utilization. We have previously reported a case of phenotypic heterogeneity on the uptake of the amino acid methionine by *Lactococcus lactis*, where two subpopulations rely each on a methionine transporter with different affinities for the amino acid (Hernandez-Valdes et al., 2020). Since one of the subpopulations primarily utilizes a high-affinity transporter, the cells in this subpopulation are likely fitter in environments with minimal amounts of methionine than the subpopulation that relies on a low-affinity transporter. Notably, this heterogeneity is apparent at the colony level, and therefore it is a remarkable example of phenotypic switching with a low rate. A further study to confirm whether this heterogeneity is a bet-hedging strategy is envisioned. The low switching rate offers the possibility to subject each subpopulation to evolution and selection experiments.

Based on the seasonal changes (e.g., light and temperature), the presence of nutrients in the environment fluctuates (Kearns et al., 2016; Mello et al., 2016; Paver & Kent, 2017). A study on *Saccharomyces cerevisiae* shows that the *GAP1* gene is responsible for adapting the population to simultaneous changes in the environment (Møller et al., 2013). Due to a hub-switch in the *GAP1* gene, three genotypes are observed: a chromosomal *GAP1* gene, an extrachromosomal circle *GAP1* gene, and the *GAP1* deletion. *GAP1* encodes an amino acid transporter for L- and D-amino acids, but participates in the pathway for stress-protecting sugar trehalose and participates in biofilm formation (Donaton et al., 2003; Torbensen et al., 2012). Therefore, different fitnesses are observed for each subpopulation in specific growth conditions. For instance, the *GAP1* deletion has lower fitness than the wild type when grown in L-glutamate as a nitrogen source, but it has a higher fitness when grown in either ammonium or allantoin. Interestingly, the presence of *GAP1* in extrachromosomal circles resulted in a higher fitness than the wild type in an evolved population with L-glutamate as a nitrogen source, probably due to an increase in gene number.

3.4 | Bet-hedging for carbohydrate utilization

In contrast to amino acids, the link between carbon source utilization and bet-hedging has been extensively described in previous studies (Gasperotti et al., 2020; Grimbergen et al., 2015; Siegal, 2015). The carbon catabolite repression in *E. coli* explains its preference for glucose and how the presence of this sugar prevents the use of other carbon sources so that only when no glucose is present, the other sugars can be utilized (Kremling et al., 2015). Recent work provides extra insight into the mechanism underlying the transition from glucose to other carbon sources in *E. coli* (Kotte et al., 2014). Kotte et al. (2014) show that during this carbon source transition, characterized by a lag phase, the population of cells is diversified into growing and nongrowing phenotypes. The subpopulation that uses the secondary available carbon source is responsible for the lag phase, whereas the nongrowing subpopulation enters a dormant state. Moreover, these subpopulations are distinguished by different glycolytic rates during their initial growth on glucose, a cell with a low glycolytic rate becomes the subpopulation that enters dormancy. The authors propose that the cells hedge their bets during their growth on glucose, resembling a conditional bet-hedging mechanism, where it occurs only after glucose is depleted, and the cells with a high glycolytic rate can cope with the change of carbon source.

Likewise, our group reported the diauxic shift from glucose utilization to cellobiose in *L. lactis* (Solopova et al., 2014). In this case, the transition between both states is determined by catabolite repression and the stringent

response. The cells that utilize cellobiose are cells that switch during the release of catabolite repression and activate the stringent response to enable cellobiose utilization.

An interesting bet-hedging mechanism related to avoiding glucose repression in *S. cerevisiae* has been described (Garcia et al., 2016; Jarosz et al., 2014). The yeast *S. cerevisiae* generally consumes glucose, preferably even when other carbon sources are present (Pinu et al., 2014). Previous studies have reported that some strains switch to a metabolic generalistic type despite their characteristic metabolic specialization, allowing them to consume other carbon sources even when glucose is present. Remarkably, this capacity relies on the inheritance of an altered protein conformation—a prion named [GAR+]. The prion is transduced from mother cells to daughter cells at different rates, depending on the nutrients in the environment, suggesting a bet-hedging mechanism to cope with the fluctuations of carbon sources. Furthermore, bacteria are able to induce [GAR+] through the production of lactic acid in such a way that strong and weak variants of the prion are observed, depending on the different concentrations of lactic acid (Garcia et al., 2016).

4 | DISCUSSION AND OUTLOOK

4.1 | Does phenotypic heterogeneity always benefit a population?

An environment where resources, especially nutrients, are constantly changing is a scenario in which microorganisms can develop different phenotypes to increase their survival chances (Childs et al., 2010; van Boxtel et al., 2017) (Figure 4). Although many studies have demonstrated how phenotypically variable bacterial strains are able to thrive in a niche, showing successful outcomes of using a bet-hedging strategy, few studies have studied the adverse effects of heterogeneity in bacterial phenotypes (Levy, 2016). In this regard, do microorganisms always benefit from displaying heterogeneity?

Troselj and Wall (2018) investigated the population response of the soil bacterium *Myxococcus xanthus* when a subpopulation of cells are starving (auxotrophic for amino acids; Troselj et al., 2018; Troselj & Wall, 2018). While no

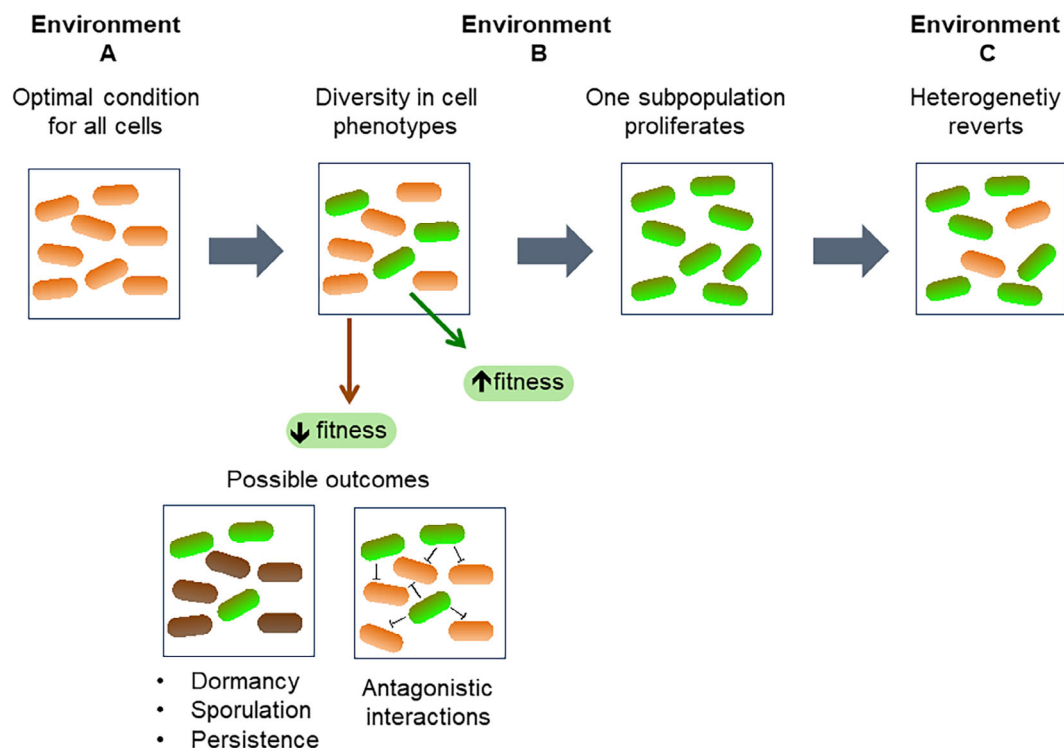


FIGURE 4 Nutrient fluctuations and cell consequences during a bet-hedging strategy. Bacteria develop phenotypic heterogeneity during changes in environmental conditions (Environment A to B), and a bet-hedging strategy results in subpopulations of cells with different fitness. While cells with low fitness are subjected to different outcomes (e.g., sporulation), the fitter cells thrive. Eventually, (change to Environment C) the cells display the diversity in phenotypes by a random switch

bacterial interaction occurs in a mixed population of prototrophic and auxotrophic cells in rich media, an antagonistic interaction is established in a starvation medium. A toxin produced by the prototrophic cells kills the auxotrophic cells, via the type VI secretion system (T6SS). An analysis of the mechanism underlying this antagonism between sibling cells shows that starving cells have lower amounts of immunity factors than the growing cells. Thus, this observation suggests that growing cells eliminate the less fit cells of the population. Several scenarios have been suggested for the biological implications of this interaction. For instance, it might be that growing cells obtain nutrients from the lysed cells, and therefore, the population becomes fitter without the starving cells, that is, as a homogeneous population. Another possibility is eliminating the nongrowing cells to avoid sporulation, which is a decision at the population level and an energetically expensive process. It would be interesting to explore whether similar antagonistic interactions against the less fit subpopulation are present in some known cases of bet-hedging strategies by bacteria, where studies have been focused on the exclusive benefits to the subpopulation that is favored under a specific nutritional condition.

Changes in nutrients have revealed bet-hedging and other phenotypic heterogeneity strategies (Gasperotti et al., 2020; van Boxtel et al., 2017). The importance of this evolutionary adaptation relies on the possibility to persist and thrive in niches where the availability of nutrients fluctuates (Balaban et al., 2004; Martín et al., 2019; Ratcliff & Denison, 2011). Since bacteria live in densely populated environments, and nutrient availability is expected to change, bet-hedging represents an adaptive evolution strategy that allows bacteria to cope with unstable environmental conditions.

5 | CONCLUSIONS

It has been long acknowledged that microbial populations employ bet-hedging strategies to persist in very dynamic and unpredictable habitats. In the last decades, due to their high relevance in the food industry (e.g., food spoilage caused by germination of persistent spores) and the medical field (e.g., antibiotic-resistant persister cells), endospore and persister cell formation have been particularly studied in bacteria (Balaban et al., 2004; Lewis, 2007; Veening et al., 2005). Nonetheless, despite the difficulties in identifying bet-hedging, recent research studies on phenotypic heterogeneity have provided many promising indications of bet-hedging strategies employed by a plethora of microorganisms. Current studies have shown that bet-hedging traits may concern many physiological aspects (Figure 3), which provides further evidence of how frequently microorganisms hedge their bets. Yet, more extensive, long-term evolutionary studies to assess the fitness gains in fluctuating environments are required to confirm the authenticity of bet-hedging cases described in previous classification reviews (de Jong et al., 2011; Grimbergen et al., 2015; Simons, 2011).

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Luiza P. Morawska: Conceptualization (equal); investigation (equal); resources (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Jhonatan A. Hernandez-Valdes:** Conceptualization (equal); investigation (equal); resources (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal). **Oscar P. Kuipers:** Conceptualization (equal); investigation (supporting); supervision (lead); writing – original draft (supporting); writing – review and editing (supporting).

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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