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NEWS AND VIEWS

OPINION

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When disaster strikes: Reconstitution of population density by expansion of survivors

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Abstract

Microorganisms have an assortment of stress-response mechanisms that enable them to survive in the face of environmental stresses. However, with prolonged exposures to severe stresses adaptive stress responses ultimately fail, the affected populations may suffer a massive decline. Recovery of the population density in the aftermath of a massive death is a vital task. Our recent post-stress regrowth under starvation (RUS) studies prompted us to propose RUS as an adaptation for overcoming consequences of devastating environmental disturbances. RUS should be seen as an integral process having two major aspects: the stress-induced cellular auto-decomposition and the recycling of the released nutrients. Here, we summarized what is already known about RUS and suggest a number of questions that are key to understanding the molecular underpinnings of these two operations. We also interrogate the prospect that would conceptualize the auto-decomposition as a fitness-maximizing mechanism acting with the purpose of an expedient supply of nutrients. Two further things are of special note: given that some of the RUS-defective mutants are also impaired in DNA repair, RUS can serve as an important tool for uncovering new determinants operating, in some overlapping fashion, in the protection of genome integrity; also, RUS can serve as a new angle of approach that might, hopefully, assign roles to some of those (up to ~ 30%) of microbial genes that are of unknown function. More generally, understanding post-stress reconstitution and the underlying mechanisms is a necessary (complementing) part of any comprehensive picture of how microbes cope with very harsh environmental disturbances.

KEYWORDS

auto-decomposition, recycling, repopulation, stress-response

1 | INTRODUCTION

Biological systems universally require specific, ordered and stable internal conditions and processes for their optimal growth and reproduction to be possible. On the other hand, spatial and temporal variation of external conditions is a general feature of nearly every habitat on Earth (Gillespie, 1994) so that the living systems are constantly exposed to fluctuating and often harsh external conditions that can disrupt their intracellular homeostasis. Although all living organisms face this crucial challenge, it is particularly pronounced in the case of free-living unicellular organisms. This is because most of them lack the capacity to actively migrate and due to the fact that – compared to multicellular organisms – microbes generally have a much greater cell surface area in direct contact with the surrounding environment. Thus, they needed to evolve their specific

stress-response strategies in order to survive and remain active in the face of ever changing ecosystems of the planet.

Indeed, numerous physiological mechanisms and ecoevolutionary strategies exist in microbes that enable them to counteract various types of environmental stress, including specific defence and repair mechanisms, the formation of biofilms, cysts and spores, as well as priming, cross protection, bet-hedging mechanisms, programmed cell death (PCD), and so forth (de Bruijn, 2016; Storz & Hengge, 2011). Risking an overgeneralization, survival strategies against stress and environmental changes could be divided in two main categories: (a) stress tolerance/resistance; and (b) restoration/resuscitation. The physiological acclimation mechanisms would fall into the first category and bet-hedging mechanisms and PCD into the second group. Namely, in the latter two processes the vast majority of the population may die under stress, but when the conditions are improved a small proportion of persister/surviving cells can restore the population by benefitting from dissolved organic materials (Balaban et al., 2004; Durand et al., 2016). A mass killing of microbial cells can also occur in well adapted populations when stressogenic agents surpass their inherent resistance and acclimation abilities. Simply, microbes are neither impervious to environmental fluctuations nor invincible by severe and prolonged stresses (Mason et al., 1986). If a harmful factor occurs in too great a quantity or concentration, or if a microbial population is exposed to prolonged episodes of harsh stresses, the accumulation of cellular damage exceeds the capacity to repair, such that microbial cells abundantly die and the population collapses. Hence, recovery of the population density in the aftermath of a massive death is a vital task.

For the reason of their prevalence and importance, understanding how microbes live, cope and die is of great interest in various fields of microbial research, ranging from eco-evolutionary to biotechnological microbiology i.e., from population dynamics in the wild to predicting their behaviour in biotechnological processes. Regarding their existence in nature, an overarching view would highlight that microorganisms live in complex, heterogeneous communities and colonize extraordinarily diverse habitats present on the planet. This remarkable success and diversity is mainly attributed to their outstanding metabolic abilities and to the armory evolved to combat and overcome stresses of various nature and intensity. However, we would want at the very least to suggest that any more detailed story should also appropriately acknowledge microbial frailness and motivate efforts to identify the molecular factors and cellular processes dedicated to reconstitution of microbial populations after catastrophic stresses. This would complement the existing picture of the survival strategies as well as extend the list of cellular mechanisms known to play a role in the ecological fitness of microorganisms.

Recent research efforts in our laboratory have been principally aimed at that direction (Milisavljevic & Kojic, 2020; Milisavljevic et al., 2018). The investigations were initially motivated by the desire to understand how a cell might maintain its genome integrity in unfavourable environments. This idea was practically distilled into experiments incorporating liquid holding (LH) as an assay system that essentially consists in monitoring post-stress recovery in viability of the treated cells held under non-nutrient/starvation conditions (Patrick et al., 1964; Roberts & Aldous, 1949). We reasoned that this classical assay should sufficiently simulate the environmental settings microbial cells may periodically encounter in the wild (flooding after a long period of oxidative stress caused by prolonged episode of desiccation) as to allow credible extrapolation of laboratory behaviour to natural environments. This paper can be considered an extended and more refined reflection on the concepts and experimental results (published and some unpublished) that constitute our initial achievements based on the foregoing perception and research strategy.

2 | RUS AS A MECHANISM OF RESTORING A POPULATION AFTER CATASTROFIC STRESS

2.1 | *Ustilago maydis* as an experimental system for exploration of stress-response strategies

The dimorphic fungal plant pathogen *Ustilago maydis* offers a very good experimental model for studying the molecular bases of adaptation to fluctuating environments. In its saprophytic (haploid) form it has to cope with the full range of environmental stresses (including UV-irradiation, desiccation, elevated temperatures), whereas in its parasitic (dikaryon/diploid) stage it must contend with the innate immune system of its host (maize). The fungus possesses a robust DNA repair capacity, illustrated by its resistance to large doses of UV or ionizing radiation. Although we do not possess a comprehensive knowledge that would explain this trait, it is evident from genetic studies in *U. maydis* that DNA repair via homologous recombination plays a central role. Mutations in any one of several genes that control homologous recombination result in a four to five log reduction in survivors at the doses of UV or gamma rays that caused no loss of viability in wild-type strains (Ferguson et al., 1997; Kojic et al., 2002, 2003). Owing to this capacity, populations of *U. maydis* cells could indeed be, to a great extent, rescued by recovery as an intracellular effect of repair of the harmed DNA. On the complex microbial-community level, this feature can certainly contribute to the adaptive advantage of *U. maydis* over all less resistant competitors.

However, as hinted above, a recent work done in our laboratory using LH assay allowed unanticipated aspects of the post-stress recovery to be detected which call for an appreciation of the importance of the cellular mechanism involved in nutrient recycling as an important determinant of population rescue from highly severe stresses. Namely, probing the ability of *U. maydis* to recover from heavy oxidative insults under LH conditions has revealed that the organism possesses an impressive capacity to recover from massive damage and that the reconstitution of the devastated cell populations is promoted by multiplication of the survivors at the expense of the dead and dying cells. The phenomenon has been termed "regrowth (or repopulation) under starvation (RUS)", and was examined

in considerable details including characterization of *U. maydis* mutants (*adr1*, *did4*, *kel1* and *tbp1*) which were isolated as defective in performing RUS in peroxide-treated cell suspensions (Milisavljevic et al., 2018). Since it was in principle possible that the inferences from our results were limited specifically to *U. maydis* and also peculiar to peroxide treatment, the study of this phenomenon was extended further by employing another fungal species *S. cerevisiae* and using a naturally occurring stressor, desiccation (Milisavljevic & Kojic, 2020).

2.2 | What do we know about RUS so far?

The major results of our work can be summarized as follows:

1. *U. maydis* and *S. cerevisiae* both reconstitute their populations after strong oxidative stress during LH even in non-nutrient conditions through multiplication of the survivors. Even cell populations that received such lethal doses of peroxide sufficient to reduce survival by five orders of magnitude could reconstitute almost the initial level of cell density when incubated for 72 hr in distilled water. However, *U. maydis* exhibits relative superiorities in rates and extents of regrowth. And needless to say, the *sine qua non* requirement for any scale of repopulation is the existence of survivors in the devastated populations.
2. Another key and obvious requirement for the repopulation is bioavailability of nutrients necessary to foster the growth and numerous multiplications of the surviving cells. This necessity is met, on the populational level, in a self-fuelling manner. Namely, the stress-induced damage of the treated cells causes release of the intracellular biomolecules into the surrounding medium and the correlations have been observed between lethality, leakage of cellular biomolecules and the doses of the stressors. Again, compared to that in *S. cerevisiae* the induction of leakage in *U. maydis* is much more rapid and profuse.
3. The leakage of the intracellular compounds is coincident with the auto-decomposition of the intracellular biomolecules in dead or dying cells. One point of particular interest is that the rates of decomposition are markedly different in *U. maydis* and *S. cerevisiae*. After peroxide treatment or desiccation the chromosomal DNA is abruptly and rapidly degraded in *U. maydis* but is scarcely affected in dying *S. cerevisiae* cells. This dramatic difference is intriguing and may reflect specific adaptations developed in relation to particular requirements of their ecological niches. Of note, since the mode of death was not studied explicitly, the terms "auto-decomposition" and "self-decomposition/disintegration" are used interchangeably here, as general terms to refer to any active degradative process internal to the dying cells, and independently of whether such a process is "programmed/regulated" or not.
4. The leakage products from the killed cells may have opposing biological activity. They provide an accessible and rich supply of nutrients in quantities sufficient to support the regrowth and replenishment of the populations decimated by oxidative damage. However, increasing the dose of the stressors as well as prolonging the post-treatment incubation increases the toxicity of the suspending medium, which can be overcome by increasing the ratio of undamaged to injured cells or by extending the time of LH incubation. Yet again, compared to *S. cerevisiae*, *U. maydis* demonstrates overall superiorities in effective recycling of the potentially harmful intracellular compounds; in this regard *S. cerevisiae* resembles some of the *U. maydis* RUS-mutants which were isolated as defective in performing RUS in peroxide-treated cell suspensions (see below).
5. All the marked differences that distinguish *U. maydis* from *S. cerevisiae* in their post-stress response to peroxide treatment are rather consistently reproduced after exposure to desiccation. Thus, the results indicate that the findings are reliable and significant from an ecological perspective.
6. Isolation and characterization of the mutants defective in RUS indicated that the global cellular machinery required for RUS is richly structured. Namely, the cellular factors identified by our research have already been known to play roles in growth regulation, protein turnover, cytoskeleton structure, transcription, heat shock response, endocytosis, cell-cycle regulation, and retrograde transport (Milisavljevic et al., 2018, and unpublished results). Second and perhaps of special note, some of the mutants exhibited extreme sensitivity to DNA damaging agents and to the DNA replication stressor hydroxyurea, suggesting that at least some of the cellular factors dedicated to RUS are also involved in the maintenance of genome integrity.

2.3 | Biological meaning of RUS and future directions

In the lifecycle of many microorganisms, prolonged episodes of severe stress can be prevalent so that the populations may experience enormous decline. Clearly life under stress cannot be sustained infinitely. If the exhaustive stress continues, adaptive stress responses ultimately fail, the cellular homeostasis gets irremediably lost, and the affected populations suffer a massive death. However, when the benign conditions are re-established, the remaining viable cells can reconstitute the devastated population through utilization of the nutrients derived from the dead cells. What emerges from our studies is that the repopulation is facilitated by both: (a) efficient recycling of the damaged and released intracellular biomolecules; and by (b) the stress-induced auto-decomposition of cellular components in the dying cells.

Therefore, we would assert that RUS should be viewed as an integral process consisting of these two major operations. The first operation is associated with viable cells (the survivors) and would involve a number of steps, including acquisition, processing and utilization of the leaked material. This operation represents the constructive side of the process, effecting growth and reproduction. The second operation is executed in nonviable cells; it is, obviously, a destructive process leading to dissolution of cellular integrity but

it is, at present, unclear whether it should be conceptualized as a passive process, resulting in degradation and liberation of intracellular biomolecules in an uncontrolled, haphazard fashion. In any case, it is a fuelling mechanism that contributes the nutritional resources supporting proliferation of the survivors. Although these two operations are, thus, carried out by two groups of cells each being in different physiological categories, they integrally serve the common good, i.e., the reconstitution of the devastated population. Accordingly, we suggest two groups of questions that indicate specific research problems revolving around these two aspects of RUS. The first cluster is about the factors that constitute the recycling machinery.

2.4 | Cellular capacity for nutrient recycling

Thus, the questions dealing with the first topic are primarily concerned with the cellular factors-functions-pathways needed for efficient reutilization of the leaked cytoplasmic compounds. Certainly, identifying the genes involved in the efficient recycling of the damaged and released biomolecules is key to any deeper understanding of this major aspect of RUS. So, what is the range of factors and cellular processes dedicated to this operation? Which of them are involved in the actual mechanics of the recycling? Are there factors whose role is more aligned with sensing/signaling and control of RUS? How many of them are implemented in the "normal/unstressed" growth? And, finally, the most intriguing question–do microbes employ specific (unknown) cellular factors required exclusively for the management of the toxic derivatives released from dying cells?

What makes the last question particularly provoking is that the analyses of microbial genome sequences regularly reveal that up to about 30% of genes identified are of unknown function (Antczak et al., 2019; Hutchison et al., 2016; Kämper et al., 2006). Nevertheless, for many of these genes potential homologues were found in diverse organisms so that a number of them probably encode universal proteins whose functions are yet to be characterized. Thus, these findings leave open the intellectually appealing possibility that some of these factors may, in fact, be involved in the post-stress nutrient recycling. If true, then the reason for their functions still being unknown lies simply in the fact that they have never been tested for their role in RUS. So, it is precisely this aspect of RUS that should be one of the first to receive much broader attention.

One other important aspect of RUS is that it employs some cellular factors that play related/overlapping roles in genome protection. Indeed, this stands to reason since, as indicated above, the auto-decomposition and leakage may supply not only beneficial but also harmful, (genotoxic) molecules-for instance, oxidized nucleotides – so that the survivors must cope with this challenge of energy-rich but risky (mutagenic?) compounds. Thus, the cellular ability to efficiently exploit the leaked material would ultimately hinge on the capacity to accomplish this task without being intoxicated. And this is truly important, meaning that RUS can serve as an important tool for uncovering new determinants of genome integrity that might not be readily revealed through other experimental approaches. In this connection, it is interesting to note here that the two RUS mutants that exhibited the most dramatic sensitivity to genotoxic agents were found to be defective in the gene encoding a homolog of Did4 (a nonessential protein involved in vacuolar protein sorting) (Amerik et al., 2000) and, surprisingly, in the gene encoding a homolog of TATA-box-binding protein Tbp1, an essential general transcription factor that functions in assembly of preinitiation complexes for RNA polymerases (Cormack & Struhl, 1992). So, we can then ask how broad are the connections between RUS activities and genome protection? What are molecular players and cellular mechanisms underpinning these overlapping processes? Are these factors also needed for overcoming oxidative pressure to which *U. maydis* is exposed during its parasitic stage?

Lastly, we may ask methodological questions of what are the most expedient approaches for identifying these cellular factors and for dissecting their respective roles in performing RUS? Thus far, we have taken a classical molecular-genetics approach. We devised a screen in which we mutagenized cells, allowed them to form colonies, and tested these individually for loss of ability to regrow in the suspension of peroxide-treated cells. This was a labour intensive and monotonous endeavor but we managed to isolate more than a dozen of RUS defective mutants. Additional mutants will be sought to help define the mechanistic basis of RUS. We believe that an unbiased mutant screen provides an unparalleled approach to survey the genome for genes involved in RUS. Simply, there is the potential to unveil hypomorphic alleles of essential genes with unexpected roles such as the possible role of Tbp1 in DNA repair. It is important to emphasize that such mutants would be missed by screening deletion collections. In the long term we believe that this approach will reveal and define many steps involved in the RUS cellular processes.

The depicted approach could be speeded up by the use of transcriptomics. If the elements of the recycling machinery are inducible they could be detected by comparing transcription profiles. Then, the genes highly induced by substrates derived from treated cells could be mutated, using gene replacement technology, and the mutants subsequently tested for their recycling ability. In conclusion, investigating the RUS process – although labour-intensive – has the potential to unveil functions of genes without functional annotation or uncover additional functions of known genes involved in genome protection and, perhaps, in cellular auto-decomposition. Which brings us to the next topic.

2.5 | The self-decomposition issue

This aspect of RUS promotes its own cluster of questions: What is the proximate cause of leakage induced by stressogenic treatments? Does the cell death (evaluated by the inability to reproduce) mean that all cell functions are diverted and uncontrolled? Is the ongoing damage process due to destruction of normal cellular organelles/compartments/etc., leading to ongoing oxidative damage, or is the

release of cellular hydrolases (acting promiscuously?) actually the real cause of the biomolecule degradation? Are there cellular factors specifically called into play under those conditions? It would also be of interest to know the chemical nature of the RUS inhibitory chemical/s, including whether they are mutagenic. Also, the question of why the toxicity of the treated cell suspensions could not be completely transferred to the derived cell-free supernatants (Milisavljevic & Kojic, 2020; Milisavljevic et al., 2018) remains to be elucidated.

However, there are a few broader issues that deserve careful attention and analysis. The first, and perhaps most important, is the question of the correlation between the type and intensity of the exposure and the mode of cellular death elicited by the insult. Although this correlation has not been studied systematically in *U. maydis* the same emerging rules that could be deduced from sparse data obtained in other microbial systems are likely to apply in this organism, too. Namely, there is evidence that stressogenic factors can produce different types of cell death depending on the nature and intensity of the stressor (Ding et al., 2012; Jimenez et al., 2009; Vavilala et al., 2015), so that apoptosis and necrosis – like in multicellular, higher eukaryotes – may represent just the extremes of a continuum of intermediate modes of cell death (Papucci et al., 2004). Since it has been recently demonstrated (Mukherjee et al., 2017) that *U. maydis* can undergo apoptosis-like cell death on exposure to strong oxidative stress, the task of further analytic clarification of the relationship between the type and intensity of stress and the resulting form of cell death seems even more inviting.

Nevertheless, evidence from our comparative studies indicated that the stress-induced cellular disintegration is carried out in a species-specific manner. Namely, under similar experimental conditions (the same type of stressing factor and upon similar level of the inflicted insult/killing) *U. maydis* and *S. cerevisiae* decompose their macromolecules in a remarkably different manner (Milisavljevic & Kojic, 2020). So, the challenge will be to determine whether or not these species-specific differences draw from different underlying mechanisms of biomolecule degradation in the dying cells. In any event, this distinctive species-specific variability in auto-decomposition observed in these two evolutionarily diverged fungal species is intriguing as it may indicate that the process has been molded in relation to specific requirements of their ecological niches. Compared to *S. cerevisiae*, *U. maydis* decomposes its intracellular molecules with markedly faster rate, and this was most apparent in the case of genomic DNA. So, has *U. maydis* evolved the capacity to rapidly decompose intracellular components for the sake of speedier supply of the nutrients? Faster auto-decomposition and release of the cellular material would, certainly, have an adaptive value especially in a nutritionally poor environment and under competition from other microorganisms. There is also an obvious ecological or efficiency benefit to this mode of dissolution of nonviable biomass. Namely, the surviving cells are gaining the nutritional benefits from the damaged, nonviable cells with a reduced cost of producing hydrolytic enzymes that would be otherwise required of them to accomplish the job. Thus, owing to self-disintegration, the benefits are imparted by decomposed organic compounds, basically donated by dying cells so that the process could, in essence, be viewed as a means for facilitating the export of fitness to the surviving subpopulation (for the fitness transfer point see Durand et al., 2019).

Therefore, faster self-decomposition makes evolutionary sense, but it is intriguing that a mechanism can be molded by selection even when its positive fitness effects are exerted through the contribution of dying cells. That is, if the auto-decomposition is an adaptation for promoting faster multiplication of the survivors then it must have been selected for; but how would the implied selection process work? In fact, this conundrum is endemic to the evolution of all biological phenomena that involve inherent, actively driven forms of death, but particularly accentuated in the case of microbial PCD (Pepper et al., 2013). Indeed, given that in unicellular life the cell is the organism, death (whether programmed or incidental) kills the entire organism, thus excluding direct selective pressure for any trait, including PCD itself. So, how could such traits evolve at all in unicellular organisms? Several adaptive and nonadaptive hypotheses for the origin of PCD in unicells have been proposed, and the reader is referred elsewhere for an extensive discussion (Durand et al., 2016, 2019; Durand & Ramsey, 2019; Nedelcu et al., 2011). One way to explore the possible adaptive nature of fast auto-decomposition would be to test the following expectation: microorganisms that evolved in fluctuating environments with prolonged episodes of harsh stress should exhibit a faster self-disintegration than those microbes evolved in more benign and stable environments.

In summing up this section, we would once again emphasize that microbial death certainly matters, yet adding in the same breath that how microbes die matters even more. Namely, in addition to the issues discussed above, it is noteworthy that the manner in which a unicell dies directly impacts the fitness of its neighbours. In a direct PCD versus non-PCD fitness comparison in *Chlamydomonas reinhardtii*, the unidentified products freed upon PCD allowed conspecific cells to grow larger and produce more offspring, whereas compounds released from cells that died immediately upon subjection to sonic waves were harmful to cells of the same strain (Durand et al., 2011). Interestingly, subsequent experiments showed that the fitness advantages of *C. reinhardtii* are species specific and that the PCD-products of this strain inhibit the growth of other *Chlamydomonas* species (Durand et al., 2014). Our experiments in *U. maydis* indicated that the compounds deriving from the treated cells become more toxic not only with the increased doses of the stressor but also with the prolonged post-treatment incubation (Milisavljevic et al., 2018). In this context, the ability of microbes to repopulate and persist in hostile environments would be expected to be directly dependent upon the ability to process the products of cellular disintegration, the toxicity of which would be influenced by type of stressors and the mode of cellular death. Therefore, the comparatively superior efficacy of *U. maydis* in the recycling of the damaged and released compounds may have played an important role in the occupation of the arid ecosystems of central America which are supposed to be centre of origin for *U. maydis* (Stukenbrock

& McDonald, 2008). Of course, this form of argument would gain more traction when the issue involves comparative study of a greater number of microbes. Accordingly, it would be very interesting (and instructive) to learn more about decomposition and leakage of the cellular biomolecules in the fission yeast *Schizosaccharomyces pombe* for which LH studies have revealed a decreased survival as a result of post-irradiation incubation in a non-nutrient medium (Harm & Haefner, 1968; Shahin et al., 1973). The effect was termed as "negative liquid holding effect" and, importantly, a more pronounced decrease in post-treatment survival for increasing doses of irradiation was reported (Shahin & Nasim, 1973).

In summary, a key task for the future will be to determine the correlation between the type and intensity of the stressor and the form of cell death as well as how this relates to both the release of the cellular biomolecules and the ability of the surviving cells to grow and replicate on these substrates. Also, comparative studies related to the ability of diverse forms of microbes to recycle the products of various modes of death appear, precisely in this context, much needed.

2.6 | The survivors

As already noted, a critical requirement for the repopulation is the existence of survivors that would re-establish the devastated population when favorable environmental conditions resume. But, who exactly are the survivors and what are some of the underlying factors that may account for the likely survivors in a harsh/fluctuating environment?

First, it is widely recognized that stressing factors such as desiccation, temperature, UV-radiation, etc. are not uniformly distributed throughout the whole habitat so that they are not homogeneously affecting populations. Therefore, even after long episodes of highly strong stress one could still expect survivors in the micro-areas where cells are subjected to a lower level of stress. Second, it is also well recognized that local populations display high genetic variability, making it likely that some of the variants will be more tolerant to the ongoing stresses. However, there are more specific eco-evolutionary strategies that can account for the survivors and for the endurance of microbial populations in risky environments. One of them is the phenomenon known as persistence (Van den Bergh et al., 2017). Namely, in clonal populations of bacteria, there is, at any moment, a small fraction of cells that are nondividing phenotypic variants of the wild type. These cells are known as "persister" cells because they persist in the face of catastrophes such as antibiotic treatment. Thus, when a genetically homogeneous microbial population of bacteria is exposed to a lethal dose of an antibiotic, the vast majority of the population dies but the rare, persister cells survive. Subsequently, the persister cells can switch to the regular, fast-dividing growth, thereby restoring the population after removal of the antibiotic (Balaban et al., 2004). The phenomenon occurs widely across bacteria, but also in yeasts (Bojsen et al., 2016; Li et al., 2015), and it has been proposed as a mechanism evolved to allow populations to avoid eradication in inhospitable environments.

A final consideration will comment on the possibility of the survivors in the context of PCD. Yordanova et al. (2013) found evidence that PCD in *C. reinhardtii* generates factors that can make the still-living cells less susceptible to the stimuli that induce death. So, it can reasonably be expected that the process eventuality ensures some survivors that can repopulate. Also, the work on PCD in *Peridinium gatunense* showed that the demise of the bloom of this dinoflagellate is orchestrated in such a way that upon sensing environmental change, some individuals in the population submit to PCD and emit a thiol esterase, which mediates a coordinated population-wide collapse by sensitizing others in the bloom to the environmental cue (Vardi et al., 2007). The reason for this behaviour has not been elucidated, but it has been speculated that the scenario allows some of the individuals in the population to survive and repopulate producing the following year's bloom (Durand et al., 2016). Lastly, in *S. cerevisiae* it was found that cells in aged yeast cultures undergo apoptotic death with concomitant release of intracellular substances that appear to stimulate the survival of other old cells (Herker et al., 2004). Interestingly, besides the requirement for the nutrients released by the dead cells, this "adaptive regrowth" is also dependent on mutations that accumulate during aging (Fabrizio et al., 2004). So, the mutants are the likely survivors who benefit from this – programmed death/adaptive regrowth – strategy that allows yeast populations to overcome periods of starvation.

3 | CONCLUSION

Microbes in natural habitats are subject to various stresses that suppress their ability to grow and reproduce so that a fundamental question in microbial ecology is how microorganisms cope with unfavorable environmental conditions. The past three decades have witnessed an explosion of new knowledge about the stress-response mechanisms, where elucidation of certain aspects of them are constantly being discovered. To this plethora of protective-stress responses we add an adaptation that is of a different kind but which complements further the picture of microbial survivability. Thus, RUS is not a stress-protective mechanism specifically developed to directly counter the corresponding stressor but a stressor-unspecific adaptation that enables restitution of viability in the aftermath of a highly killing stress. After exposure to massive environmental stress leading to a drastic decrease in population size, the remaining viable cells can reconstitute the devastated population through utilization of the nutrients derived from the dead cells. Given that the repopulation would be contingent upon the recycling of the released organic materials, the essence of the adaptation must primarily lie in the cellular preparedness for such an operation.

However, our studies have indicated that faster auto-decomposition rate is also an important contributor to the efficacy of the repopulation. As noted above, *U. maydis* owes its relative superiority in performing RUS not only to its remarkable ability

to reuse the released organic materials but also to the evidently faster decomposition and leakage of the cellular molecules. Thus, faster self-disintegration may be particularly relevant in facilitating adaptation to a nutrient-poor environment. The benefits are, in a devastated population, imparted by released organic materials so that rapid decomposition may enhance the expansion pattern of the surviving subpopulation. Yet, reaping the benefits would, again, depend on the ability of nutrient recycling. Thus, given that the repopulation is an outcome of the combined actions, RUS should be seen as an integral process consisted of these two major operations.

The exploration of RUS is in its infancy so that, as with many other initial studies, our investigations too provide more questions than answers. Indeed, we now require a global understanding of the range of factors and biological processes underpinning RUS, as well as how these processes are coordinated to bring about efficient reconstitution of populations. We argued that finding the genes involved in the recycling mechanism is key to understand RUS as an adaptation that may be crucial for enabling many microorganisms to persist in nature. It is likely that new cellular factors connecting RUS and genome protection have yet to be identified, and understanding the mechanisms and coordination of these processes leaves many exciting questions for the future. All in all, considering the plenitude of the listed (and related) questions, it is clear that answering them will require extensive further experimentation. Also, as the research moves further on, it is likely that the ongoing endeavour and advances will be accompanied with a conceptual expansion upon these existing issues. It is our hope that this contribution will help fuel some of these advances by offering a new prism to view the field and providing a theoretical framework of ideas and critical "next-step" questions for further studies. RUS represents a certain widening of perspective and insight that can only enrich our exploration and understanding of the microbial survivability in very harsh natural environments.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Both authors contributed to the initiation and further development of the conceptual framework presented in this paper as well as to the writing of the manuscript.

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