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Cannibalism as cell differentiation meant that *Bacillus subtilis* NRS-762 is not suitable as model organism in survivability studies of microbes

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Abstract

Survival of microbes on various surfaces and environment is a question of importance to basic science, as well as health care, water treatment and distribution, ecology, and search for life in other planetary bodies. To this end, various model organisms, known to be resilient against a variety of environmental insults are used for understanding the mechanisms underlying survival in extreme environments, or conditions mimicking those of the investigated habitats. Serendipitous observations of drastic decline in optical density of *Bacillus subtilis* NRS-762 (ATCC 8473) in LB Lennox and Tryptic Soy Broth (TSB) at temperatures of 25, 30 and 37 ^{o}C, after the aerobic culture reached maximal cell density at stationary phase, pointed to possible cell lysis as mechanism for cell death. Specifically, optical density of the bacterium declined from 5.4 at 22.5 hours post inoculation in LB Lennox to 2.5 after 38 hours of culture at 25 ^{o}C and 250 rpm rotational shaking. Similarly, optical density of *B. subtilis* also precipitously declined from 6.4 at 33 hours of culture to 1.8 at 51 hours post inoculation at 37 ^{o}C in TSB. This is in stark contrast to aerobic growth of *Escherichia coli* DH5α (ATCC 53868) in LB Lennox at 37 ^{o}C and 250 rpm, where optical density remained stable during stationary phase. More importantly, observations of *B. subtilis* culture after autoclave decontamination revealed lack of cellular debris; thereby, indicating massive cell lysis resulting in population collapse. Although *B. subtilis* is known to enter into various cellular differentiation programmes upon nutrient starvation such as onset of stationary phase in cell culture, complete absence of cell debris that usually settle at the bottom of the shake flask after autoclave decontamination, pointed to cannibalism or prophage induced cell lysis as key reasons underlying observed drastic decline in optical density of the culture. Specifically, prophage induced cell lysis may be discounted as this would have destroyed the entire cell population expeditiously shortly after entry into stationary phase. Hence, cannibalism, where a subpopulation of *B. subtilis* cells secrete cell lysis factors which other *B. subtilis* cells are not resistant to, likely result in massive cell lysis that generated cellular contents that could serve as nutrients for the surviving cell population resistant to the cell lysis factors, and may be the dominant mechanism underpinning observed rapid decline in optical density after entry into stationary phase. Collectively, *B. subtilis* NRS-762 is not suitable as model organism for microbial survivability studies given its tendency to undergo differentiation into the cannibalism programme, which in killing a significant fraction of cells upon nutrient deprivation, would also confound experiments aimed at understanding the resilience of cells towards various extraneous environmental factors not common in the microbe's favoured habitats.

Keywords: cannibalism, prophage, cell lysis, *B. subtilis*, model organism, optical density, drastic decline, viable cell population, cell differentiation, resistant population,

Subject areas: microbiology, biochemistry, biotechnology, molecular biology, cell biology,

Conflicts of interest

The author declares no conflicts of interest.

Author's contribution

The author read a *Scientific Reports* paper describing work utilizing *Bacillus subtilis* for assessing the survivability of microbes, in general, under conditions mimicking those currently known to exist on Mars. Having gained some experience working with *Bacillus subtilis* NRS-762 (ATCC 8473) in his masters' research, where population collapse was observed in microbial culture after the culture reached stationary phase, the author does not suggest the use of *Bacillus subtilis* NRS-762 as model organism for assessing microbial survival under conditions mimicking those of other planetary bodies, beyond or within the Solar System, or other test scenarios such as assessing whether the bacterium could survive on surfaces of pipes carrying drinking water.

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