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INTRODUCTION

Volatile compounds are known to mediate signaling between bacterial species as well as between insect and bacteria. Interkingdom signaling between flies and *Proteus mirabilis* is known to activate swarming motility in the bacterium; thus, suggesting a link between nutritional stress and induction of cell survival response. Herein, a serendipitous observation of flies gathering on or moving around the cotton plugs of aerobic shake-flask cultures of *Bacillus subtilis* NRS-762 together with drastic decline in optical density and absence of cellular debris after autoclave decontamination was synthesized into a coherent conceptual model. Specifically, interkingdom signaling may be part of a two-pronged strategy by which *B. subtilis* cope with fluctuations in environmental stressors occurring across a continuum of timescales, where, volatile compounds attract flies as transport vectors in an “proactive response,” while activation of different cellular differentiation pathways such as sporulation, competence, cannibalism etc. serve as a bet-hedging strategy for “in situ” adaptation. Interested researchers are invited to extend upon the preliminary findings reported.

OBSERVATIONS

- Drastic decline in optical density of *B. subtilis* NRS-762 after entry into secondary phase (Fig. 1) coincident with observation of flies on and around the cotton plugs of aerobic shake-flask cultures maintained on an open orbital shaker at 25 °C.
- Presence of flies co-occurred with the emanation of odour from stationary phase cultures, and the number of flies positively correlated with odour pungency.
- More intense attempts at gaining entry into the cotton plugs by flies were observed with greater odour pungency, which suggested possible behavioural modifying effects of the volatile compounds.
- Lack of cellular debris after autoclave decontamination suggested that sporulation, if initiated, did not progress to a significant extent. More probably, cell lysis was initiated across the population by an unknown mechanism.

WHAT COULD HAVE HAPPENED?

- Upon entry into stationary phase, *B. subtilis* secreted volatile compounds (Fig. 2), which attracted flies as possible transport vectors for long-range dispersal to more favourable ecological niches.
- Secretion of volatile compounds could be cell density dependent; in particular, greater nutritional stress and smaller cell population induced the secretion of more volatile compounds. The concept is analogous to a “SOS” response.
- Higher concentration (and pungency) of volatile compounds helped attract more flies to the cultures, and more intense “foraging.”
- Induction of cell lysis in a subpopulation via cannibalism could be one way by which *B. subtilis* attempted to prolong population survival. Release of cytoplasmic content and pre-synthesized volatile compounds could account for greater pungency observed.

Fig. 1: Growth of *Bacillus subtilis* NRS-762 in LB Lennox medium at 25 °C and 250 rpm.

Fig. 2: Concept map illustrating how nutrient depletion could simultaneously activates secretion of volatile compounds as interkingdom signaling molecules, and cell differentiation in *B. subtilis*.

Proposed two-pronged cell survival response in *Bacillus subtilis* for coping with environmental fluctuations occurring on a range of timescales

Fig. 3: Long-range dispersal to new locales via transport vectors complements *in situ* responses (e.g., spore formation and cannibalism) in a two-pronged survival strategy, which may have a deep evolutionary origin in the domain, Bacteria.

CONCEPTUAL MODEL

- In response to environmental stressors, *B. subtilis* might have secreted volatile compounds for attracting and enlisting flies as transport vectors in long-range dispersal to more favourable ecological niches, in what may be termed as a “proactive” approach (Fig. 3).
- In parallel, various cell differentiation pathways such as cannibalism, competence and sporulation might be initiated. High energy cost of sporulation meant that it is the option of last resort. Through secretion of cell lysis toxins, cannibalism likely help prolonged the survival of the population through release of cytoplasmic nutrients that sustain remaining cells.
- Declining cell population might trigger further secretion of volatile compounds by remaining cells as a desperate measure for attracting flies to help disperse a subpopulation to a new habitat to increase the survivability of the clonal population.
- Broadly, volatile compound secretion and interkingdom signaling may constitute an *ex situ* (proactive) response useful for responding to potential irreversible decline in a microcosm’s habitability. It might operate in tandem with “static” survival response tuned for coping with short- to medium-term environmental fluctuations.
- Together, the two-pronged approach provided bacteria with a complete repertoire for adapting to stressors occurring on a range of temporal scales, and may have an ancient evolutionary origin.

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