

## Fate of pathogenic *Bacillus cereus* spores after ingestion by protist grazers

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The aim of this study is to understand the symbiosis between bacterivorous protists and pathogenic bacterial spores, in order to gain insight on survival and dispersal of pathogenic bacteria in the environment. It is generally accepted that resistance to grazing by protists has contributed to the evolution of *Bacillus cereus* group bacteria (e.g. *B. cereus*, *B. anthracis*, *B. thuringiensis*) as a pathogen. It has been hypothesized that the spore stage protects against digestion by predating protists. Indeed, *B. thuringiensis* spores have been shown to be readily ingested by ciliated protists but failed to be digested (Manasherob et al 1998 AEM 64:1750-).

Here we report how diverse protist grazers grow on both vegetative cells and spores of *B. cereus* and how the bacteria survive ingestion and digestion, and even proliferate inside the digestive vacuoles of ciliated protists. The survival ability of *B. cereus* was initially investigated in microcosms inoculated with pure cultures of the protists *Acanthamoeba castellanii*, *Tetrahymena pyriformis* and *Cercomonas* sp. as grazers. Individual protist cultures were fed with fluorescently labelled (CellTracker™RedCMTPX) *B. cereus* spores or vegetative cells as the only food source. The presence of fluorescently labelled intracellular bacteria confirmed that *B. cereus* spores as well as vegetative cells were ingested by protists and appeared intact when observed by epi-fluorescence microscopy. Secondly, *B. cereus* digestion and protist growth were determined by qPCR and protists appeared to grow on spores, though they grew better on vegetative cells. Finally, *B. cereus* spore germination was observed inside the ciliated protist *T. pyriformis* after antibiotic treatment of the protist surface which seems contradicting to the observed protist growth on spores. Initially these observations indicate that protists might act as a survival niche and potential breeding ground for *B. cereus* with some loss of bacteria to support growth of the protist. This indicates tight symbiosis between bacteria and protist grazers and will be discussed.