Mechanistic and transcriptomic salt stress effects on *Bacillus subtilis* spore germination and outgrowth

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The spore-forming soil bacterium *Bacillus subtilis* frequently encounters changes in environmental conditions within its natural habitat. Flooding, desiccation, and agricultural activities can, for instance, cause strong fluctuations in osmolality. While the complex osmotic stress response of vegetative cells is increasingly understood, less is known about salt stress effects on germinating and outgrowing spores, which, however, is of applied interested for soil ecology, food microbiology, and astrobiology. Although negative effects of high salinity have been reported, they have been poorly investigated on a mechanistic level in the past. Thus, we have investigated the effects of NaCl and other salts on spore germination and outgrowth of *B. subtilis* and could gain interesting new mechanistic, structural, and transcriptomic insights.

While the initiation of germination is still possible despite high salinity, increasing NaCl concentration cause an increasing delay of germination commitment, increasing heterogeneity of germination onset, slower germination on single spore and population levels, and decreasing germination efficiency. Data from broad analyses using various techniques suggest NaCl-inhibition of at least one early process in germination (e.g. commitment or germination initiation), and of at least one subsequent event. The most likely processes to be inhibited are ion, Ca^{2+}-DPA and water fluxes, which might be negatively affected by direct interactions of Na^+ and Cl^- with transporters or channels, annihilation of a chemical gradient, and/or low extracellular water activity. We have strong evidence that NaCl-inhibition is due to a combination of ionic and osmotic effects. Moreover, the spore coat plays an important role for successful germination at high salinity, likely being involved in protection from ionic stress. Outgrowth, defined by the onset of metabolism after germination, can also be detected at high salt concentrations (≤ 4.8 M NaCl), although the ability to proliferate at high salinity strongly depends on the composition of the germination medium. To determine underlying physiological and genetic mechanisms, the salt stress response of outgrowing *B. subtilis* spores was investigated using RNA-seq. While the transcriptomic salt stress response during outgrowth revealed similarities to the response of vegetative cells, we could also identify several interesting differences.