Intracellular pH Response to Weak Acid Stress in Individual Vegetative Bacillus subtilis Cells

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Supplementary Data

Video S1. Growth of *B. subtilis* PptsG-IpHluorin vegetative cells in defined minimal (MOPS-buffered) medium (pH 6.4). The video shows three movies of respectively the phase contrast image as well as the fluorescent emission images upon excitation at 390 nm and 470 nm.

Video S2. Growth of *B. subtilis* PptsG-IpHluorin vegetative cells in defined minimal (MOPS-buffered) medium (pH 6.4) containing 3 mM potassium sorbate. The video shows three movies of respectively the phase contrast image as well as the fluorescent emission images upon excitation at 390 nm and 470 nm.

Video S3. Growth of *B. subtilis* PptsG-IpHluorin vegetative cells in defined minimal (MOPS-buffered) medium (pH 6.4) containing 25 mM potassium acetate. The video shows three movies of respectively the phase contrast image as well as the fluorescent emission images upon excitation at 390 nm and 470 nm.

Table S1. Results obtained from Multichannel-SporeTracker of growth of *B. subtilis* PptsG-IpHluorin vegetative cells at single cell level. Exponentially growing *B. subtilis* PptsG-IpHluorin vegetative cells were inoculated in defined minimal (MOPS-buffered) medium (pH 6.4) supplemented with (**A**) nothing (control), (**B**) 3 mM potassium sorbate and (**C**) 25 mM potassium acetate. Note that as was observed previously by van Beilen et al. (figure 1 b in ref. 4) pH_i may start at values above 8 likely indicating a stalled metabolic activity of the (control) cells at the start of imaging. Growth-rate and average

24 colony pH_i calculations with Multichannel-SporeTracker were always performed from
25 the time-point where discernible surface increase ,i.e. growth, had resumed. pH_i values at
26 the start of our observations differed between both batches of *B. subtilis* cells shown.