

Presence of Calcium Lowers the Expansion of *Bacillus subtilis* Colony Biofilms

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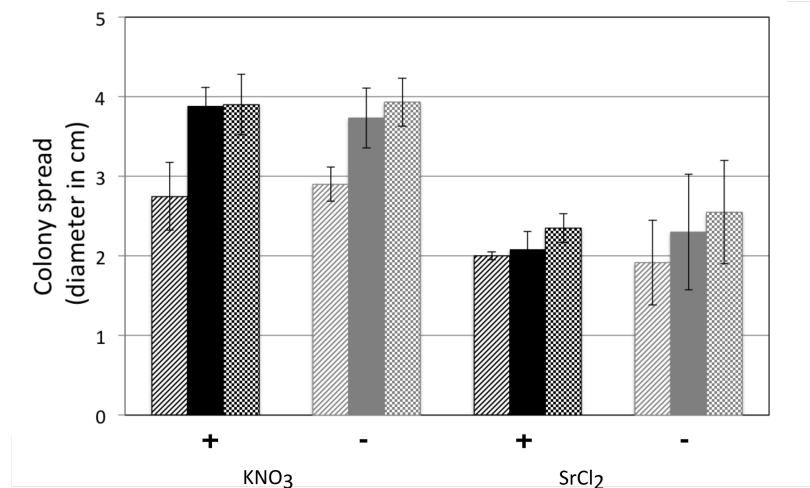


Figure S1. Ca²⁺ specifically reduce colony escape. The colony expansion diameters of *B. subtilis* DK1042 are shown after 3 (striped), 5 (filled), and 7 (checked) days. Black bars present data in presence, while grey bars indicate in absence of KNO₃ and SrCl₂ in the 2×SG medium. KNO₃ was used to observe the impact of NO₃⁻, while SrCl₂ was applied to assay the influence of another divalent cation. The error bars indicate 95% confidence intervals.

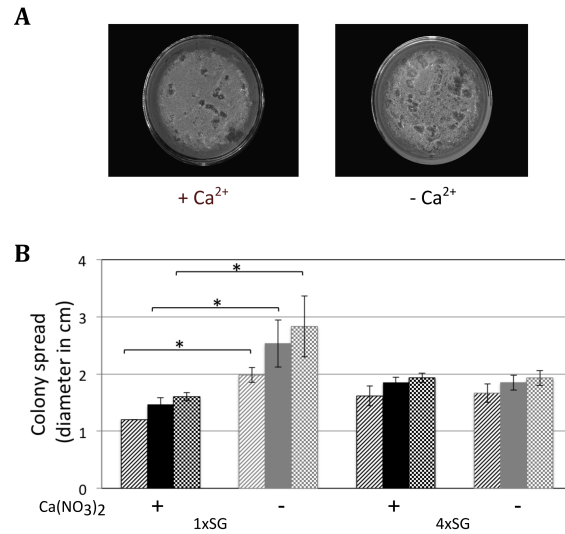


Figure S2. Impact of Ca²⁺ on pellicle formation and colony spreading at different nutrient concentrations. (A) Pellicle formation of *B. subtilis* DK1042 on 2×SG medium in the presence (left) and absence (right) of Ca²⁺ supplementation after 3 days. (B) The colony expansion diameters of the *B. subtilis* DK1042 are shown on 1×SG and 4×SG media with 1.5% agar after 3 (striped), 5 (filled), and 7 (checked) days. The black bars present data in presence, while grey bars indicate in absence of Ca²⁺ supplementation in the media. The error bars indicate 95% confidence intervals. * denotes significant differences (p<0.05) analyzed with paired t-test.

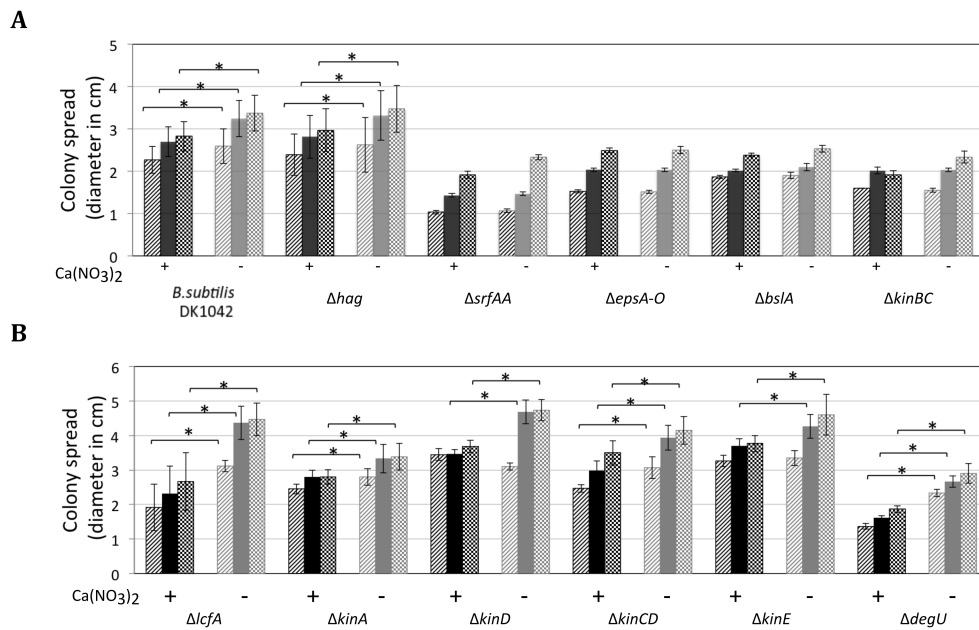


Figure S3. Colony escape of various strains on MSgg (A) and 2×SG (B) medium. (A) The colony expansion diameters of the *B. subtilis* DK1042 and its derivatives, Δ*hag*, Δ*srfAA*, Δ*epsA-O*, Δ*bslA*, and Δ*kinB*Δ*kinC* are shown on MSgg medium after 4 (striped), 6 (filled), and 8 (checked) days. (B) The colony expansion diameters of the *B. subtilis* Δ*lcfA*, Δ*kinA*, Δ*kinB*, Δ*kinC*, Δ*kinD*, Δ*kinE*, Δ*kinC*Δ*kinD*, and Δ*degU* are shown on 2×SG medium after 3 (striped), 5 (filled), and 7 (checked) days. Black bars present data in presence, while grey bars indicate in absence of Ca²⁺ supplementation in the respective media in both panels. The error bars indicate 95% confidence intervals. * denotes significant differences (p<0.05) analyzed with paired t-test.