## Importance of bacteria-mineral associations for biofilm development and gene transfer efficiency

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Soil includes a range of different surfaces compositions and characteristic which can attract different bacteria for induction of bacterial communities, the so-called biofilms. Biofilms protect cells from stress and provide a niche for horizontal gene transfer (HGT) to e.g., share antibiotic resistant genes (ARG) [1]. We here aimed to address the effect of different soil minerals on two soil-habitat bacteria, Gram positive *Bacillus subtilis* 168 and Gram-negative *Acinetobacter baylyi* BD413 and investigated (1) Bacterial cell survival and membrane integrity of two bacterial strains, (2) The ability of bacteria to take up circular DNA preadsorbed to mineral surfaces through HGT and (3) Biofilm development on minerals using metabolic activity assay, Scanning electron microscopy (SEM) and Optical Photothermal Infrared (O-PTIR) Spectroscopy.

We find that mineral surface structure, surface charges, and wettability play a major role in attraction or repulsion of bacterial cells and that both bacteria got injured when encountered to highly positively charged nano minerals.

Our results confirmed that bacteria through HGT can incorporate DNA adsorbed to the mineral surfaces and that the rate of DNA transformation was related to mineral types. Hydrophilic negatively charged and hydrophobic positively charged minerals resulted in the highest number of transformants, demonstrating that the transformation is independent on mineral charge and mineral wettability.

SEM images showed that cell clusters were formed on positively charged surfaces while scattered adherences were observable for negatively charged surfaces (Fig. 1). O-PTIR was done to investigate whether the substrate influenced matrix composition and supported the idea that the intensity of the matrix production of biofilms on positively charged surfaces was higher than the rest of the minerals.

The current study demonstrates that bacterial physiology underwent changes by the mineral type and characteristics.

[1] Fleming and Wuertz, Nat Rev Microbiol, 2019



Fig 1. Biomass and matrix production is mineral dependent. a) Biofilms attachement and no biofilm structure on negatively charged mineral surface.