Surface thiol sites of *Bacillus subtilis* are associated with detrital proteins

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Introduction: Bacterial surface proteins that contain the amino acid cysteine harbor a thiol functional group side chain. Recent spectroscopic investigations have revealed that the cell surface thiol functional groups on the gram positive bacterium *Bacillus subtilis* are involved in metal binding. Although the entire complement of proteins expressed by *B. subtilis* has been characterized, the publically-available proteomic data have yet to be incorporated into a geochemical framework to model metal complexation. In this study, we examined the *B. subtilis* proteome to identify thiol-containing proteins on the bacterial cell surface. The results of this work elucidate the first steps toward integrating proteomic data into a surface complexation model to describe bacterial metal binding.

Materials and Methods: A catalogue of cysteine-containing proteins in the *B. subtilis* proteome and their predicted sub-cellular localization was compiled. Laboratory experiments were conducted to measure the concentration of proteins adsorbed on the cell surface during growth. The abundance of thiols associated with the adsorbed proteins was quantified.

Results and Discussion: We found that *B. subtilis* cell wall proteins contained no or very few cysteine residues. Also on the cell surface were over six hundred proteins that were distinct from cell wall binding proteins. Many of these proteins were highly enriched in cysteine. Sequence analysis revealed that these surface-associated proteins were enzymes that catalyze core biosynthetic pathways typically located in the cytoplasm. We hypothesized that during cell death, intracellular enzymes were released into the aqueous medium and this protein detritus subsequently adsorbed to the surfaces of other cells. To test this hypothesis, we conducted experiments to characterize the loosely attached proteins on the cell surface. Adsorbed proteins were detected at all stages of growth. Proteins desorbed from the cell surface contained high concentrations of reduced thiols. Desorption of cell surface proteins completely removed the cell surface thiol sites. These results indicate that surface thiol sites of *B. subtilis* are associated with detrital proteins that originate from decaying biomass and are loosely attached to the cell surface.