Investigating the role of extracellular phosphate groups in bacterial adhesion to soil minerals

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Understanding bacterial adhesion is important for solving environmental problems associated with the fate and transport of bacteria and anthropogenic contaminants in soil and water. Bacterial adhesion to soil minerals is mediated by the exterior of bacterial cells, which are comprised of surface proteins, nucleic acids, extracellular polymeric substances (EPS), lipopolysaccharides (LPS), teichoic acids, and other biomolecules (Davies, 1999). Currently the specific mechanisms by which bacteria adhere to mineral surfaces remain largely unknown. The primary objective of this research is to investigate the molecular-level interactions of bacteria during adhesion and to examine the role of surface functional groups, particularly phosphate during bacteria adhesion to iron oxide minerals. The research builds on previous efforts to understand bacterial adhesion to Fe-oxide surface (Omoike et al., 2004; Parikh and Chorover, 2006).

All chemical solutions were prepared in acid-washed vials using Barnstead Nanopure (BNP) water. The chemicals Casamino acid, Tryptone, and Deoxyribonucleic acid (fish sperm DNA) were each dissolved in 1 mM NaCl to have a concentration of 8 mM with approximately pH 7. Bacteria were grown at 25 ºC under aerobic environment in 25 mL of LB broth growth media. After 24 hours, bacteria were harvested and used in 50 mL of experimental culture. The cells were harvested by centrifugation in 3000 RCF for 20 minutes at 4 ºC and washed again with 10 mM of NaCl to remove growth media and free EPS. Bacteria were resuspended immediately in 10 mL concentration with 10 mM NaCl for FTIR experiments. 1 mL of bacteria were deposited on α-Fe_2O_3 coated ZnSe and binding observed via FTIR spectroscopy. ATR-FTIR spectra were collected using a ZnSe IRE at pH 7 with 1mM NaCl for chemical and 10mM NaCl for bacteria as background electrolyte. Spectra were obtained depositing solution onto a ZnSe crystal, and by depositing the same soltuon onto a α-Fe_2O_3-coated ZnSe crystal.

By observing changes in FTIR spectra before and after reaction with α-Fe_2O_3 information regarding the functional groups involved in binding can be acquired. Collection of spectra requires careful experimental techniques but repeated experiments demonstrate that the results are reproducible. The interpretation of spectra is challenging but initial interpretations are in general agreement with previous results (Parikh and Chorover, 2006); however the new data important information regarding the role of carboxyl groups not previously elucidated. Results demonstrate bacterial adhesion of *Pseudomonas sp.* to α-Fe_2O_3 is mediated through both phosphate (1041 cm^{-1}: Fe-O-P) and carboxyl functional groups (1350 cm^{-1}: Fe-OCO or Fe-O-C-O-Fe) (Figure 1). The use of D_2O permits verification of appropriate subtraction of water from FTIR spectra and reduces ambiguity of analysis in the amide region where overlapping OH bands from water are observed. While the binding of *E. coli* (data not shown) to α-Fe_2O_3 seems to occur by similar mechanisms, the differences in FTIR spectra indicate some divergence in binding mechanism between these bacteria. This difference likely stems from differences in biomelcular cell surface composition. The binding of model biomolecules (e.g., amino acids and DNA) show similar results (Figure 2) but since spectra are significantly different to those of living bacteria it is unlikely that DNA or these amino acids are the specific surface biomolecules in the bacteria mediated the adhesion. Further research is needed to determine the source of both phosphate and carboxyl moieties involved in bacterial adhesion.

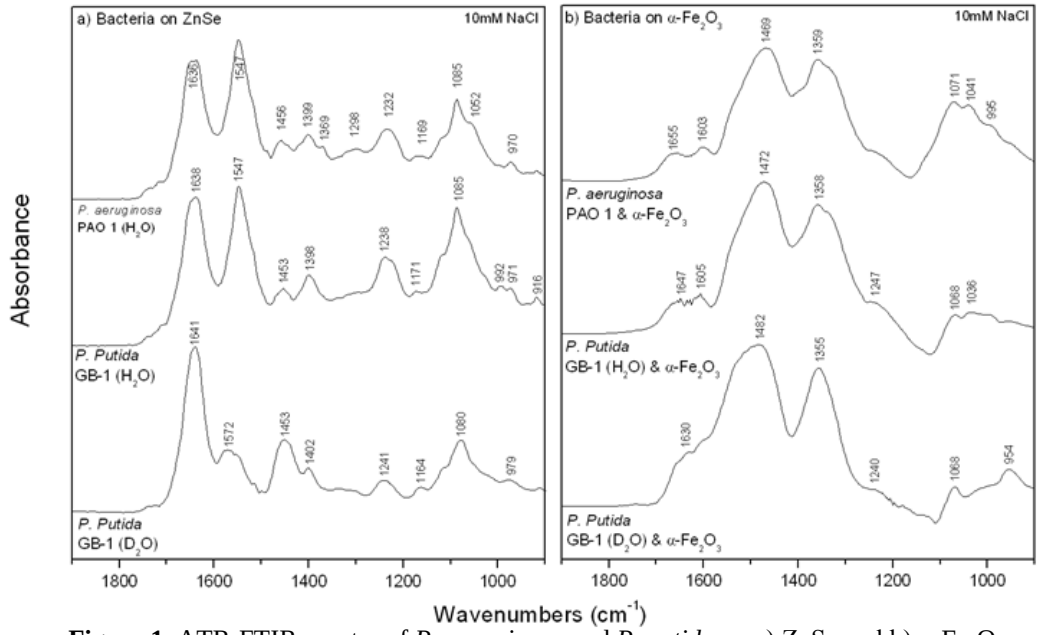

Figure 1. ATR-FTIR spectra of *P. aeruginosa* and *P. putida* on a) ZnSe and b) α-Fe_2O_3. Spectra for *P. putida* were collected in both H_2O and D_2O.

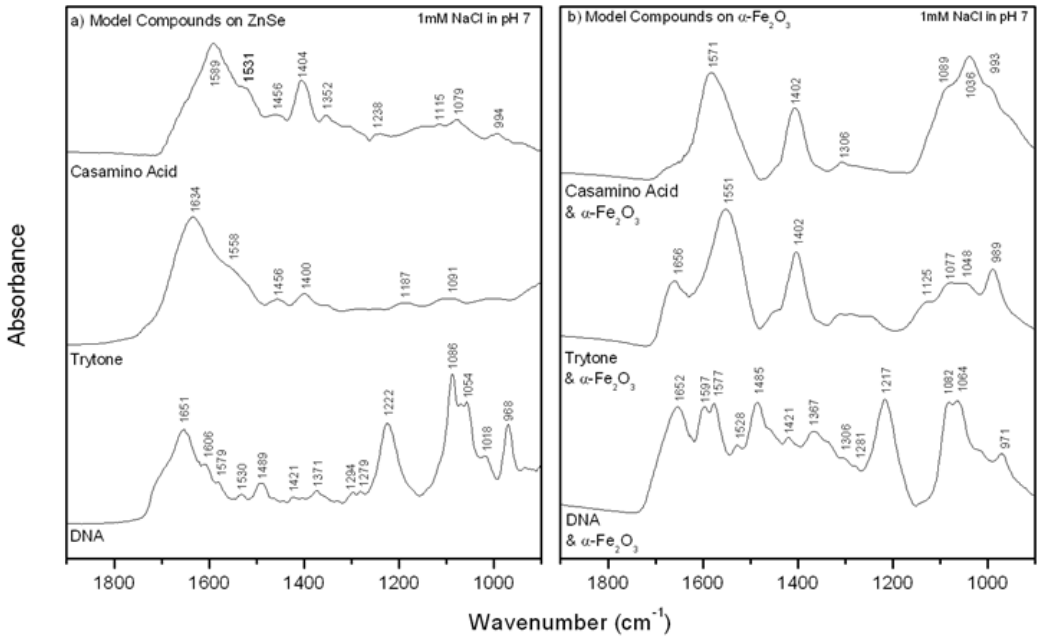

Figure 2. ATR-FTIR spectra of model compounds on a) ZnSe and b) α-Fe_2O_3.

References

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