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

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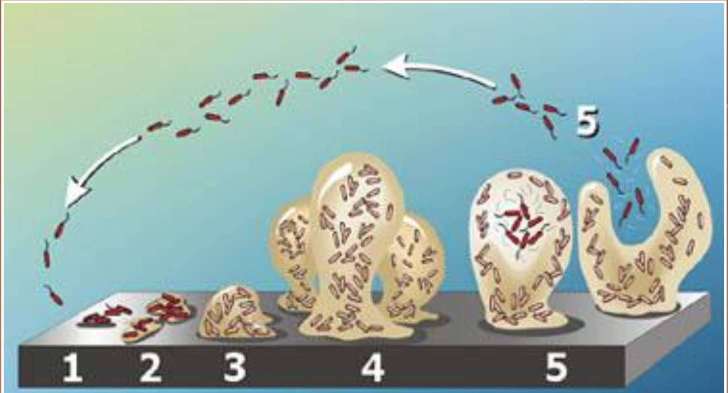
Bacterial Adhesion

- Bacterial adhesion is important for understanding biofilm formation, pathogen and contaminant transport, and bioremediation of soil and water.
- Bacterial adhesion represents the attachment of the bacteria to solid surfaces (e.g., soil minerals).
- Minimizing bacteria adhesion is important for water treatment facilities and for reducing contaminant transport in soil and water.



http://gnn.tigr.org/gnn_images/news_content/06_02/biofilms/biofilm1.jpg

Objectives

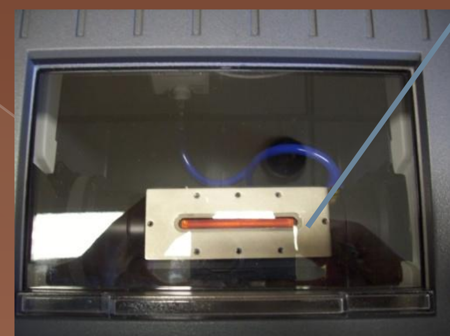
- To examine the role of functional groups within biomolecules during bacterial adhesion to Fe-oxide minerals
- To use a range of model compounds with phosphate and carboxyl groups to determine the types of biomolecules most important for bacterial adhesion to Fe-oxides

Methods

Growing bacteria

Centrifuge & wash bacteria with 10 mM NaCl

Hematite (Fe-oxide) Coating



Bacteria

Interacting

Fourier transform infrared (FTIR) spectroscopy to elucidate binding interactions between functional groups and hematite coating

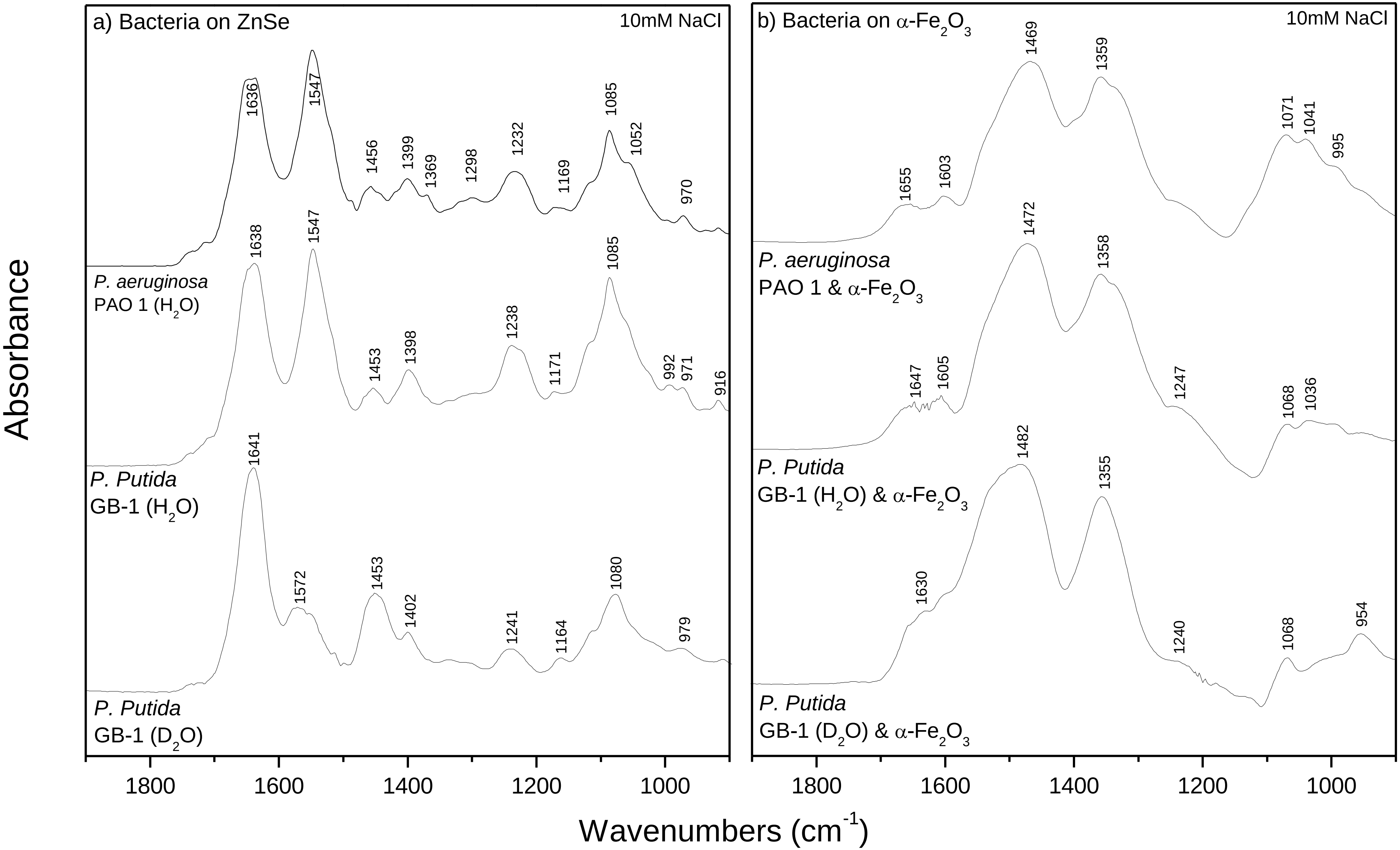
All experiments verified by running the experiment a minimum of two times.

Common IR assignments for bacteria and biomolecules

Wavenumber (cm^{-1})	IR Band Assignment
1720-1740	$\nu_{as}(COOH)$ §
1652-1637	Amide I: C=O, C-N, N-H
1570-1580	$\nu_{as}(COO^-)$
1550-1530	Amide II: N-H, C-N
1460-1454	$\delta\ (CH_2)$ †
1400-1390	$\nu_s(COO^-)$ ‡
1220-1260	$\nu_{as}(PO_2^-)$
1170	ν(C-O)
1137	$\nu_s(PO_2^-)$
1114-1118	ν(C-O-P, P-O-P), ring vibrations
1106-1108	$\nu_{as}(PO_3^{2-})^-$
1084-1094	$\nu_s(PO_2^-)$, ring vibrations, ν(C-O)
1078	ν_s(C-O-C, C-C), $\nu(PO_3^{2-})$
1048-1060	ν(C-O-C, C-C)
1042-1046	$\nu_s(PO_3^{2-})$
1039-1043	ν(P-OH, P-O-Fe)
1016-1020	ν(P-O-Fe), ring vibrations
979	$\nu(PO_3^{2-})$
974	ν(P-OH)
962-970	$\nu(PO_2^-)$

§. ν_{as} = asymmetric stretching vibration, †. δ = bending vibrations, ‡. ν_s = symmetric stretching vibration.

Pseudomonas sp.



Pseudomonas sp.

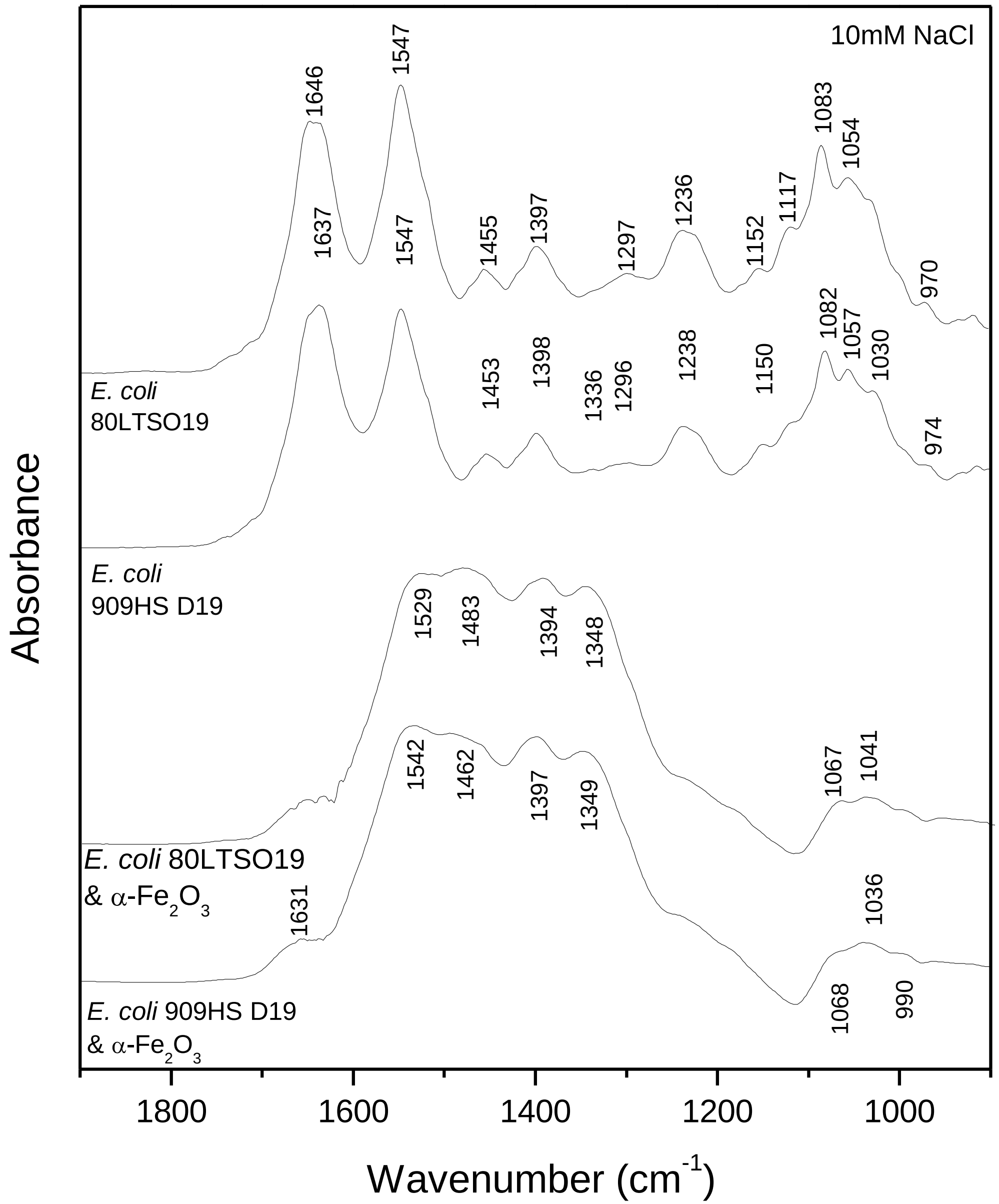
- Gram negative bacteria
  - P. aeruginosa PAO1 - wildtype
  - P. putida GB-1 – Mn-oxidizing strain

- Bacteria Analysis with D_2O
  - 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.
  - The band at ~1547 cm^{-1} arises primarily from OH, the use D_2O verfied that the subtraction when water is present is correct as few other differences are observed between spectra of *P. putida* with D_2O and H_2O.

Pseudomonas sp.

- Reaction w/ a-Fe_2O_3:
  - Carboxyl Groups:
    - The downshift and relative growth in the 1350 cm^{-1} (from 1400 cm^{-1}) indicates binding between COO^- and Fe
  - Phosphate Groups:
    - phosphate peak (PO_2^-) at1238 cm^{-1} is reduced and a new peak at ~1070 cm^{-1} is present indicating change in coordination of phosphate group upon binding to hematite
    - increase in relative contributions of phosphate (1040-1100 cm^{-1})
    - new peak at 1036-1041 cm^{-1} is attributed to formation of P-O-Fe bonds
  - Other peaks:
    - The band at ~1450 cm^{-1} is typically assigned to $CH_{2;}$ however, the reason for increase and shift of this peak upon reaction with a-Fe_2O_3 has not yet been determined

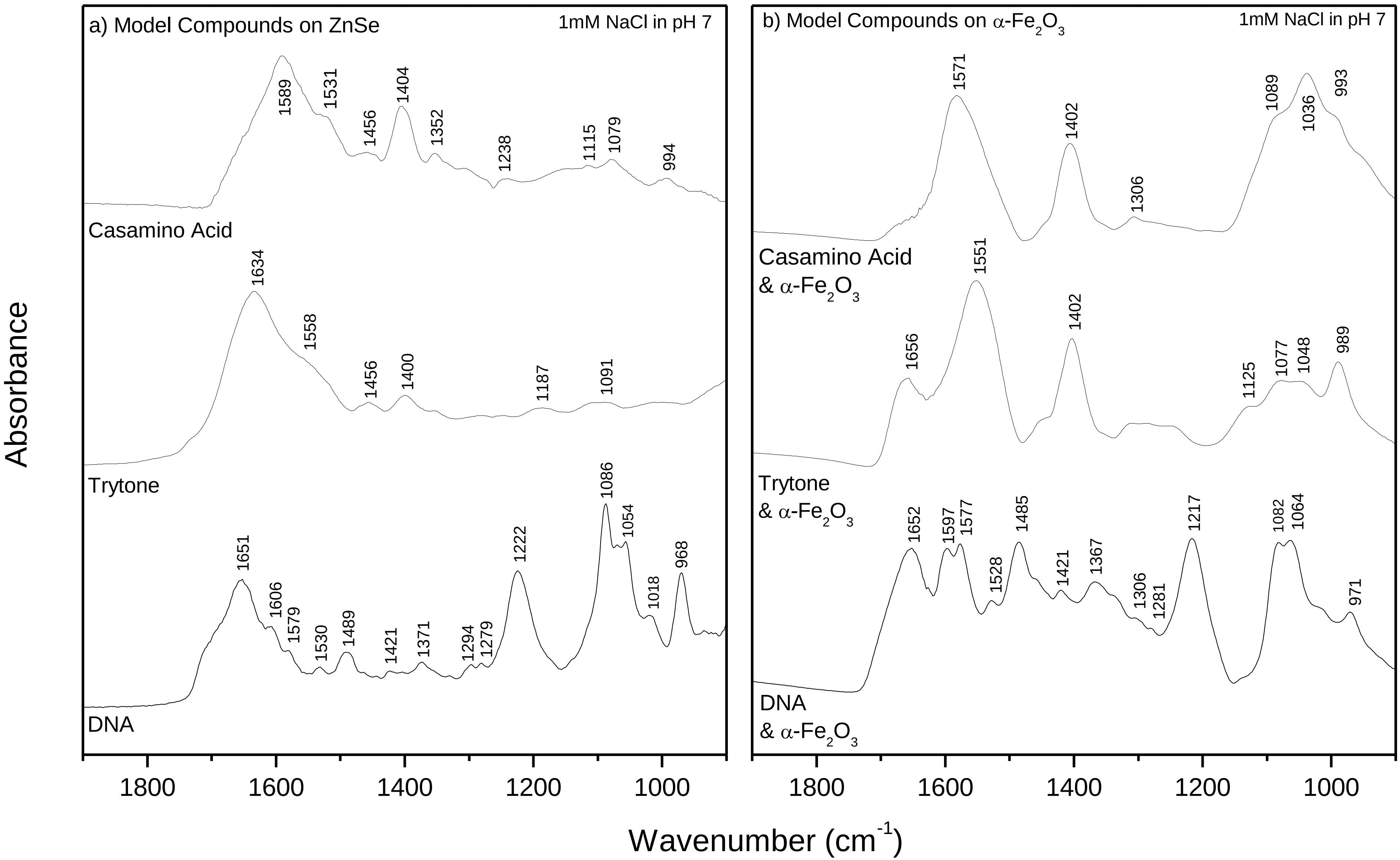
E. coli


10mM NaCl
1646
1547
1637
1547
1455
1397
1297
1236
1152
1117
1083
1054
970
E. coli
80LTSO19
1453
1398
1336
1296
1238
1150
1082
1057
1030
974
E. coli
909HS D19
1529
1483
1394
1348
E. coli 80LTSO19
& α-Fe2O3
1542
1462
1397
1349
1067
1041
1631
1036
E. coli 909HS D19
& α-Fe2O3
1068
990
Absorbance
1800
1600
1400
1200
1000
Wavenumber (cm-1)

E. Coli

- Gram negative bacteria
  - Strains: 80LTS019 and 909HS D19
  - Isolated from the California Delta
- Comparison with *Pseudomonas sp.*
  - Similarities in wavenumbers <1300 cm^{-1}
  - *E. Coli* has increased contributions at ~1540 and 1397 cm^{-1}
- Reaction w/ a-Fe_2O_3:
  - Carboxyl Groups:
    - relative growth of the peaks at 1394 and 1397 cm^{-1} and a shift from 1589 to 1571 indicates some involvement of COO^-
    - The presence of strong peaks at ~1397 cm^{-1} likely result from unbound COO-
  - Phosphate Groups:
    - phosphate peak (PO_2^-) are reduced at1238 cm^{-1} and a new peak at ~1067 cm^{-1} is present indicating change in coordination of phosphate group upon binding to hematite
    - increase in relative contributions of phosphate (1041cm^{-1})
    - new peak at 1036 cm^{-1} is attributed to formation of P-O-Fe bonds

Model Biomolecules



Casamino Acids

- Casamino Acids is a mixture of unlinked amino acids

- Casamino Acids reacted w/ a-Fe_2O_3:
  - Carboxyl Groups:
    - relative growth of the peak at 1402 cm^{-1} and a shift from 1589 to 1571 indicates some involvement of COO^-
  - Phosphate Groups:
    - phosphate peak (PO_2^-) disappears at1238 cm^{-1} and a new peak at 1089 cm^{-1} is present indicating change in coordination of phosphate group upon binding to hematite
    - increase in relative contributions of phosphate (1040-1100 cm^{-1}), compared to carboxyl and amide region, are observed upon interaction with hematite
    - new peak at 1036 cm^{-1} is attributed to formation of P-O-Fe bonds

Tryptone

- Tryptone is similar to Casamino Acid; it is a mixture of amino acids that bind through peptide bonds to form long chain polypeptides.

- Tryptone reacted w/ a-Fe_2O_3:
  - Amide Region:
    - shift of amide I occurs upon reaction with hematite (1634 to 1656 cm^{-1})
    - change in amide I:amide II (~1640:1550 cm^{-1}) indicates change in protein conformation upon binding
  - Carboxyl Groups:
    - relative growth of the peak at 1402 cm^{-1} indicates some involvement of COO^-
  - Phosphate Groups:
    - new peak at 1077 cm^{-1} (PO_2^-) and 989 (PO_3^{2-}) result from a change in coordination of phosphate group upon binding to hematite
    - increase in relative contributions of phosphate (1040-1100 cm^{-1}), compared to carboxyl and amide region, are observed upon interaction with hematite
    - new peak at 1048 cm^{-1} is attributed to formation of P-O-Fe bonds

DNA

- Deoxyribonucleic Acid (DNA) molecules are comprised of two strands of phosophdiester-containing nucelotides linked through H-bonding to form a double helix configuration.

- DNA reacted w/ a-Fe_2O_3:
  - Change in the amide region (1500-1700) results from autooxidation of DNA by a-Fe_2O_3
  - The presence of numerous peaks in the carboxyl strecthing region (~1390 to 1600) also indicate cleavage of ribose ring structures
  - The carboxyl groups from oxidized DNA likely interact with a-Fe_2O_3 to mediate binding of DNA transformation products

Conclusion

- This research demonstrates the importance of phosphate and carboxyl functional groups for adhesion of live bacteria to Fe-oxides

- The primary biomolecules on bacterial surfaces which initiate this adhesion remain unknown; however, from this and previous research it is believed that a mixture of nucleic acids, amino acids, and other biomolecules are involved.

- This research helps increases our understanding regarding the initial moments of bacterial adhesion and subsequent biofilm formation.