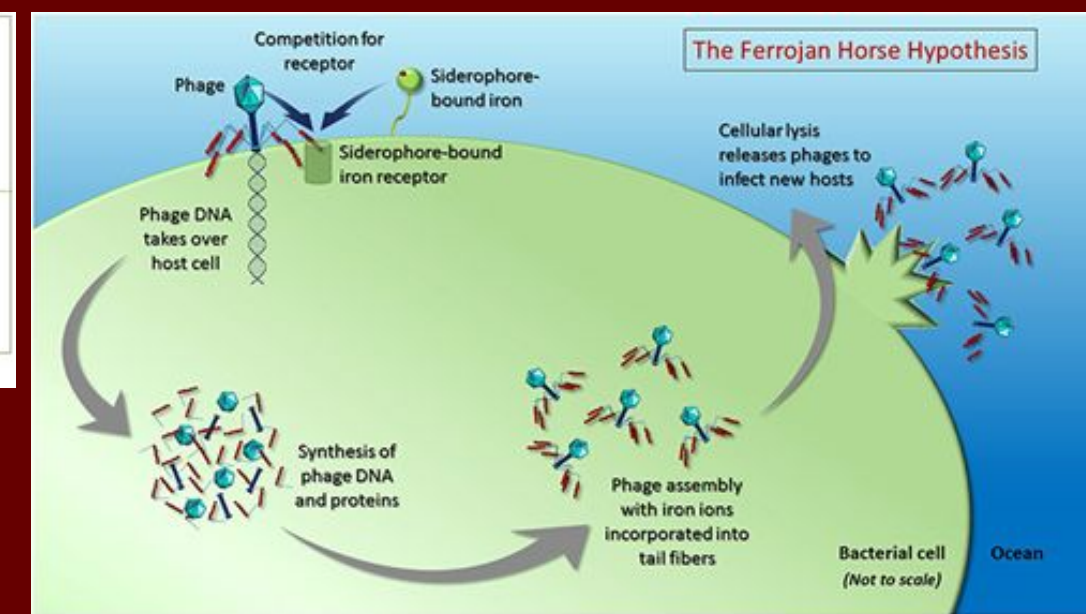
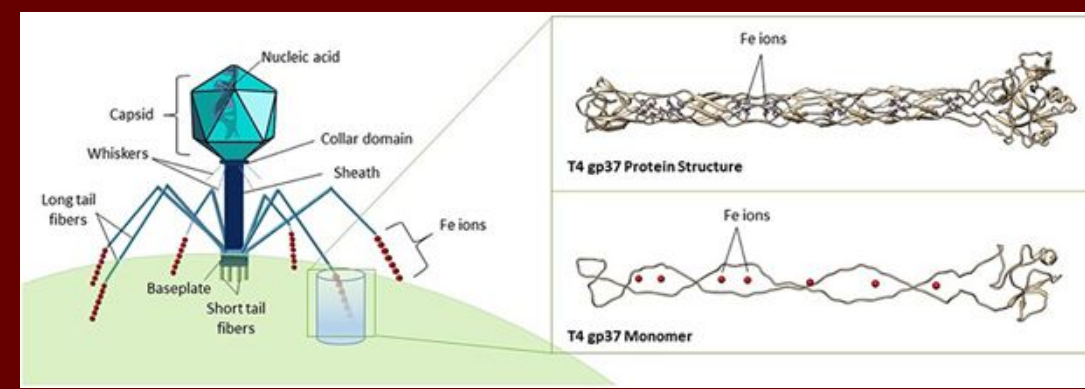


Analysis of the Role of Iron Uptake Mechanisms and Addition of Iron-Doped Apatite Nanoparticles in Phage Infection in *Staphylococcus aureus* and *Mycobacterium smegmatis*

By Linda Rost, Baker High School, Baker, MT

Background

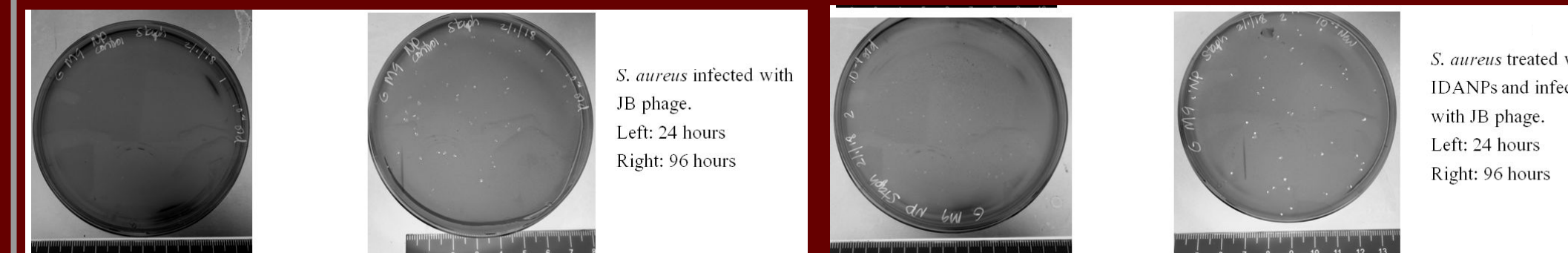
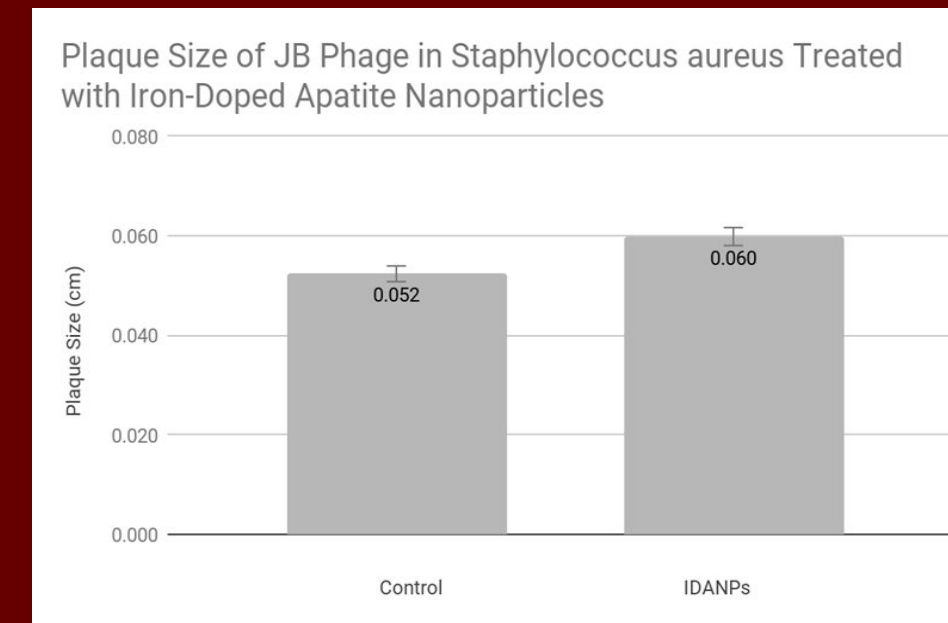
- Antibiotic-resistant bacteria have developed into a global health crisis.
- Phages, viruses that infect bacteria, could serve as an alternative to antibiotics, and have many benefits (Loc-Carrillo & Abedon, 2011).
- Iron-doped apatite nanoparticles (IDANPs), developed at MT Tech, increase phage infections (Gregory, 2017).
- The mechanism is unknown, but could involve the iron uptake mechanism of bacteria (Bonnain, Breitbart & Buck, 2016).



Reprinted from The Ferrojan Horse Hypothesis: Iron-Virus Interactions in the Ocean by Chelsea Bonnain, Mya Breitbart and Kristen Buck, 2016, *Frontiers in Marine Science*, (3). Reprinted with permission.

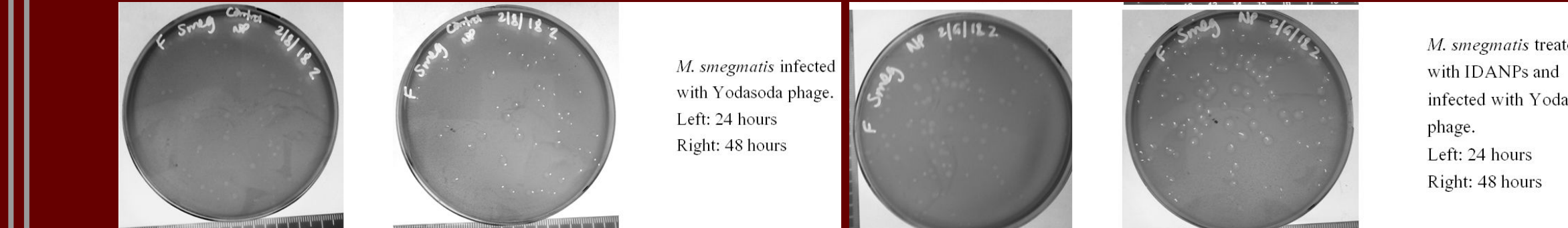
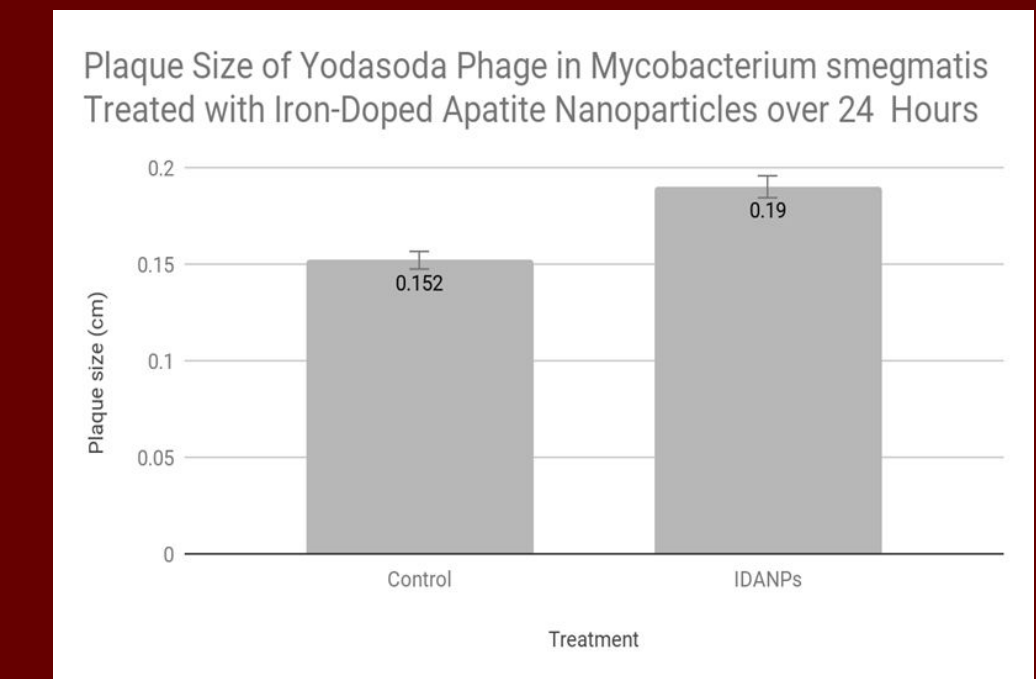
Plaque Size with IDANPs in *S. aureus*

- *Staphylococcus aureus* was grown in M9 minimal media, treated with IDANPs, and infected with JB phage.
- Plaque sizes were larger when cells were treated with IDANP.
- Plaque size 15% larger - 24 h in IDANPs vs. control; $p < 0.0001$
- 9.2% larger - 48h in IDANP; $p=0.021$
- 7.8% larger - 48h in control; $p=0.146$
- 50.8% larger - 96h in IDANP; $p<0.001$
- 39.0% larger - 96 h in control; $p<0.001$



Plaque Size with IDANPs in *M. smegmatis*

- *Mycobacterium smegmatis* was grown in luria broth, treated With IDANPs and infected with Yodasoda.
- Plaque sizes were larger when cells were treated with IDANPs.
- Plaque size 25% larger - 24 h in IDANPs vs. control; $p < 0.0001$
- 57.4% larger - 48 h in IDANP; $p < 0.001$
- 64% larger - 48 h in control; $p < 0.001$

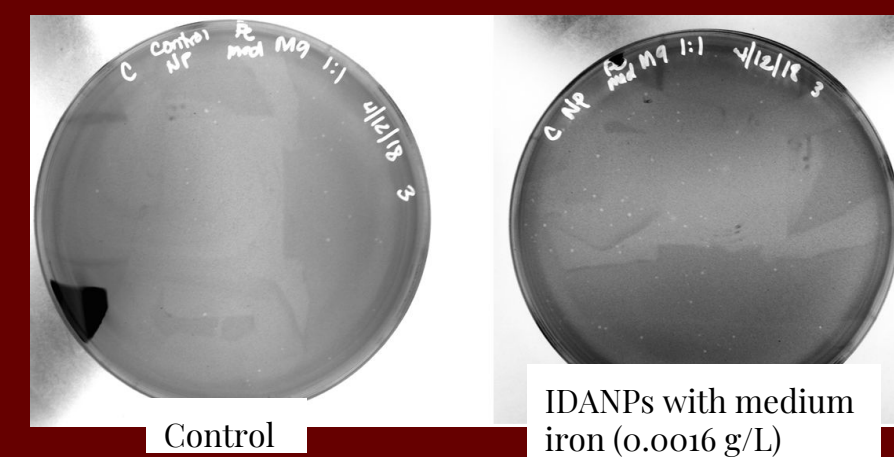
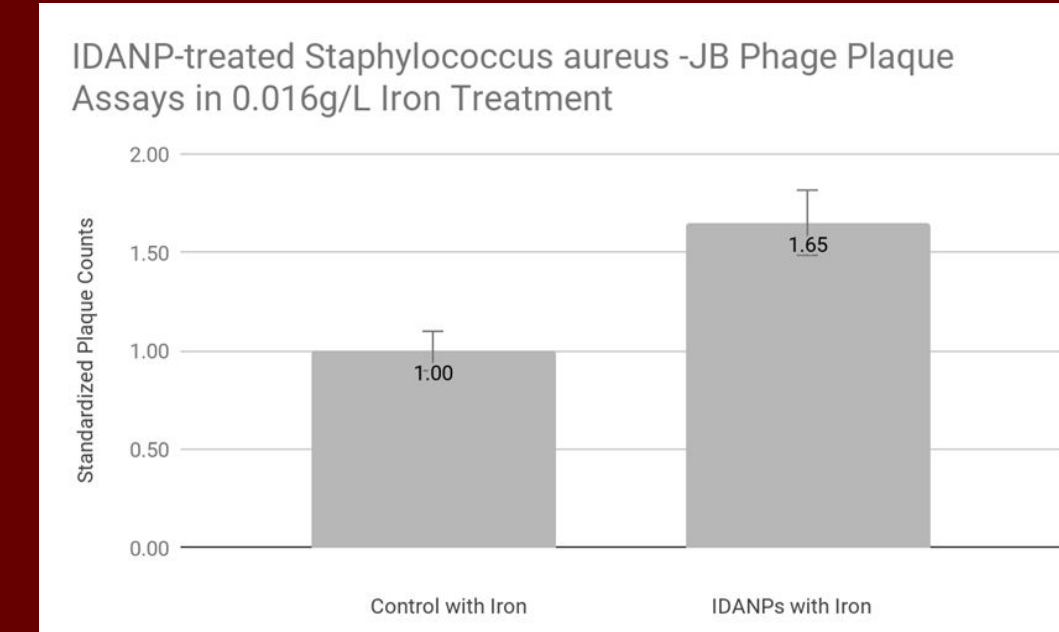


Research Questions

- Does the iron uptake mechanism of *Staphylococcus aureus* play a role in phage infection?
- Do IDANPs affect plaque size in *Staphylococcus aureus* and *Mycobacterium smegmatis*?
- What effect do iron treatments and IDANPs have on phage infection?
- How do increases in iron concentration and IDANPs affect cell growth?

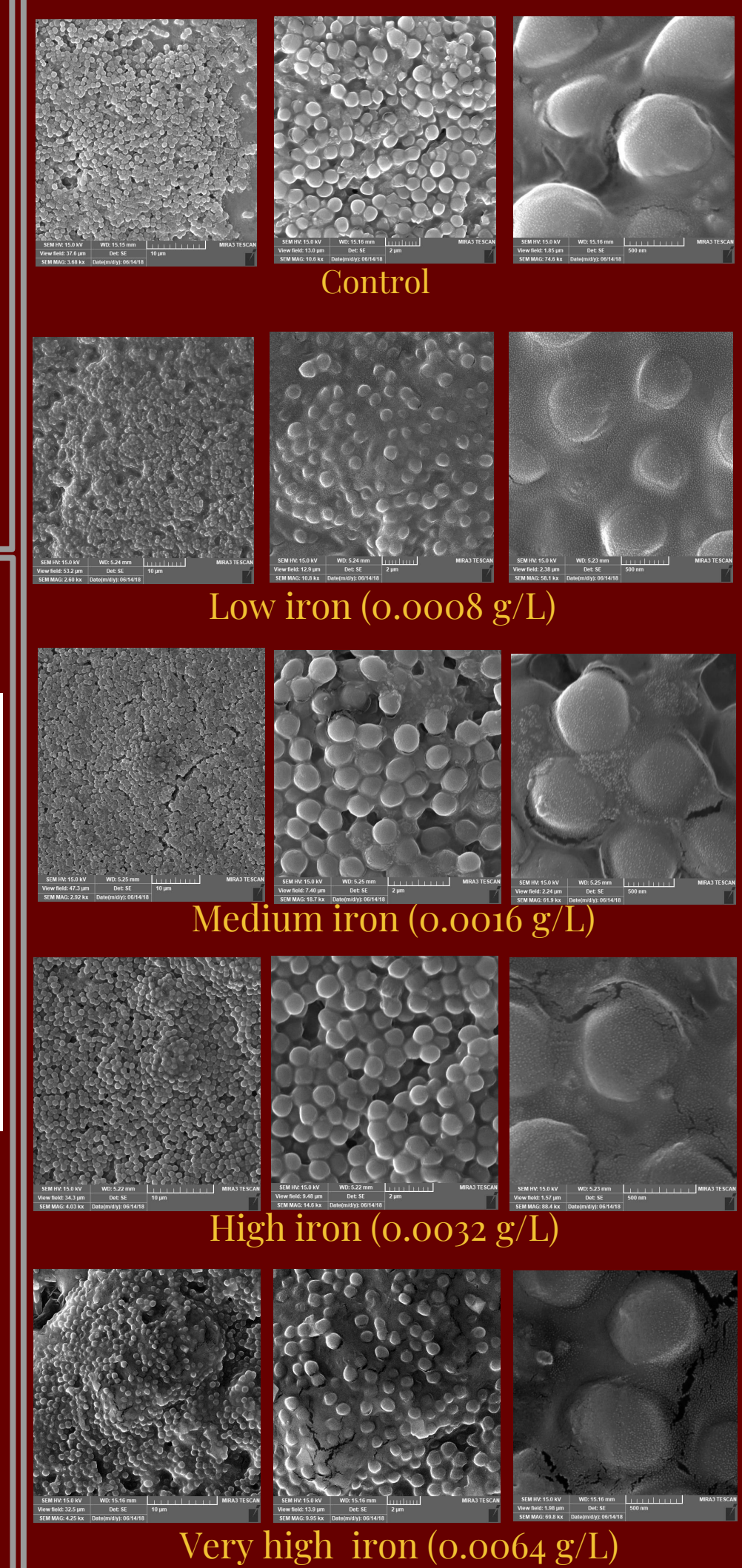
IDANPs with Iron Treatment in *S. aureus*

- *Staphylococcus aureus* was grown in M9 minimal media, treated with iron and IDANPs, and infected with JB Phage.



- Plaque counts were 65% higher in iron treatments than IDANPs alone.

SEM of *S. aureus* in Iron Treatments

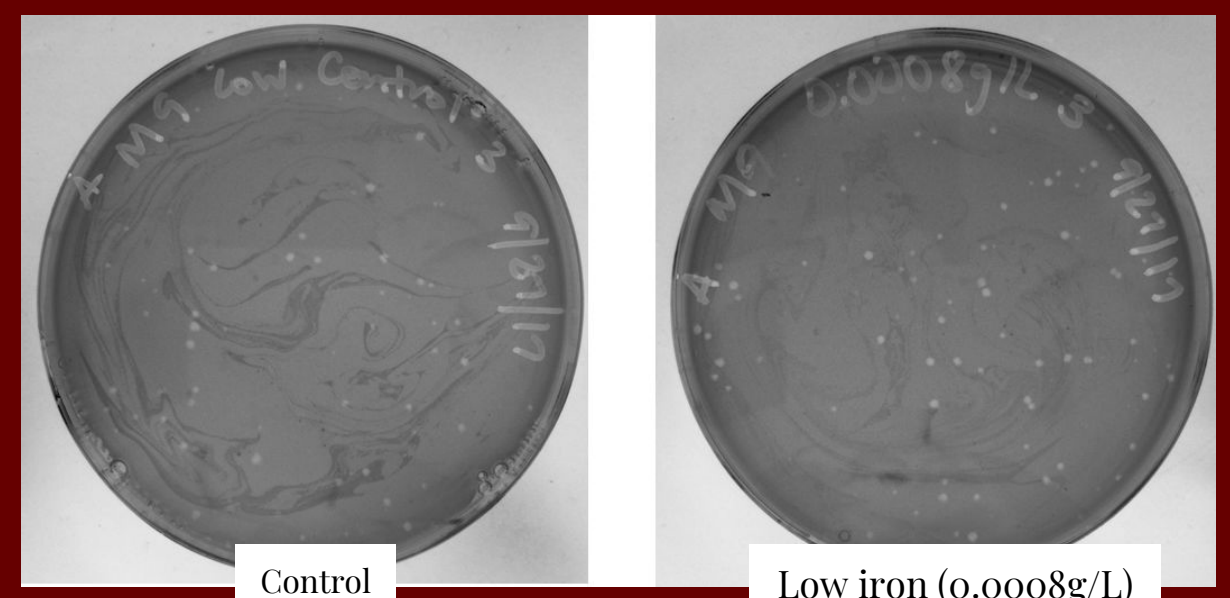
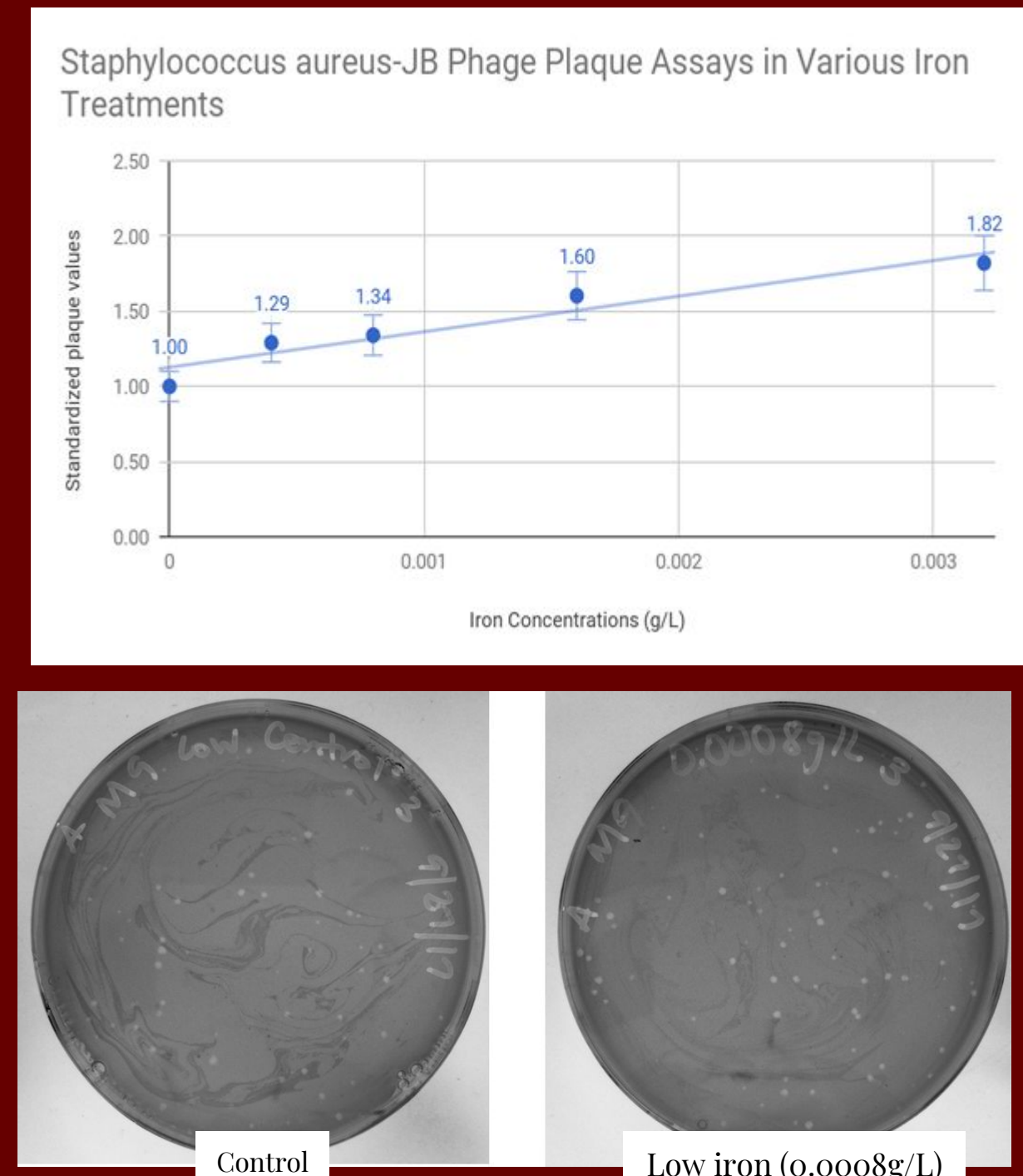


Discussion and Conclusion

- Plaque counts increase with iron concentrations.
 - Phages may be binding to iron uptake receptors.
 - Fewer are bound to siderophores in high iron treatments, leaving them available to phages.
- IDANPs increased plaque count and plaque size.
 - Plaques became larger over 96 hours in the IDANP-treatment and to a lesser extent in the control.
- Cells grown in iron and IDANPs had higher plaque counts.
- Cell growth was not significantly different with IDANPs or iron treatments, but cell counts were lower in the high iron treatments.
 - The medium iron treatment had the highest growth.
 - High iron treatments did not produce adequate lawns to perform plaque assays.
 - SEM showed cell morphologies were not different.
- IDANPs increase phage infection and plaque size in these two prokaryotic biological systems.
- Plaque count and size are important in phage therapy.
- Could elucidate the mechanism of IDANP interaction.
- The cell's iron uptake mechanism is involved in phage infection.

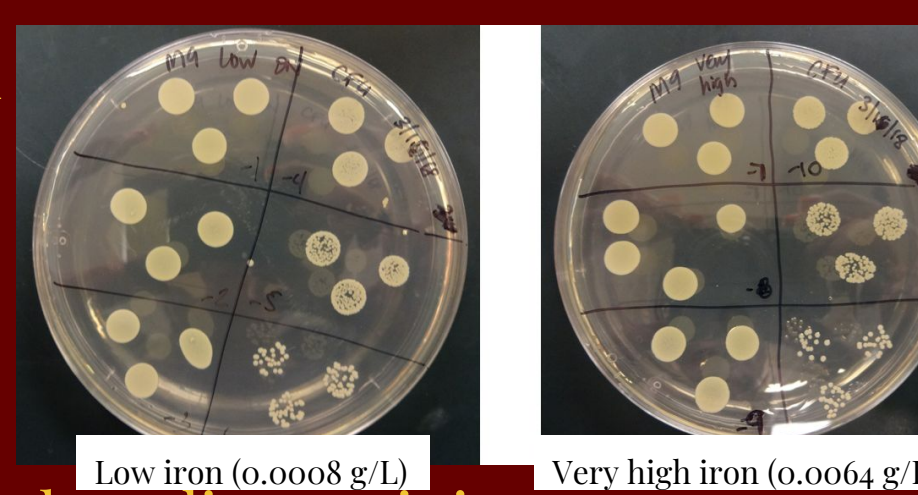
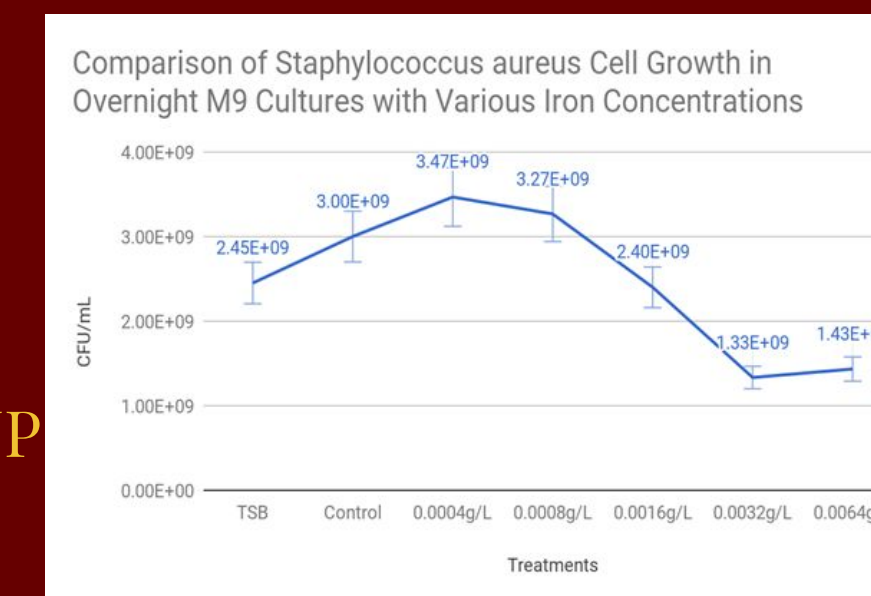
Iron treatments in *S. aureus*

- *Staphylococcus aureus* was grown in M9 minimal media, treated with various iron treatments and infected with JB phage.
- Phage infection increased with high concentrations of iron.
- $y=236x+1.13$
Where y = standardized plaque counts, x =iron Concentration (g/L)
- $p<0.0001$; $r=0.95$



S. aureus Growth in Iron and IDANPs

- *Staphylococcus aureus* was grown in M9 minimal media, treated with iron and IDANPs and CFU/ml was measured
- M9 Control: 3.33×10^6 ; IDANP: 3.92×10^6
- No difference over 3hr in M9 and IDANP
- No difference in M9 and IDANP in various iron treatments over 3h.
- Difference in M9 minimal media iron overnight treatments.
- Decreased growth in high iron.
- SEM did not show differences in morphology between iron treatments.
- Nanoscale inclusions were M9 minimal media precipitates.



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