hcnABC operon transcription of *Pseudomonas putida* under varying iron and oxygen concentrations and culture age

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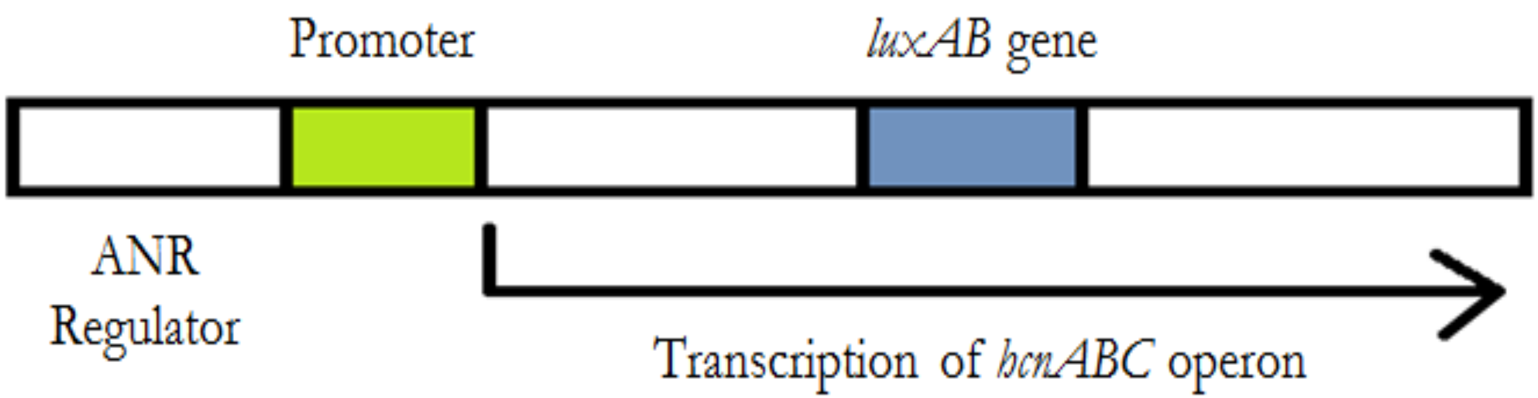
Introduction

- *Pseudomonas putida*
  - Plant-associated bacterium
  - Produces hydrogen cyanide (HCN)
  - What is HCN?
- Significance
  - HCN prevents tobacco black root
  - Suppresses growth of Velvetleaf (weed species)
  - Agricultural implications

Introduction

- Mechanism of HCN production



Transcription of *hcnABC* operon → HCN synthase → HCN production

Introduction

- HCN is lethal – why are we not affected?
- Regulation of HCN production at *hcnABC* operon
- 2 significant regulators:
  - Anaerobic regulator (ANR)
    - Low oxygen levels and/or high iron levels → Activation of ANR → Transcription of *hcnABC* operon → HCN production
  - Global activator (GacA)
    - High cell densitiy → Activation of GacA → Transcription of *hcnABC* operon → HCN production

Introduction

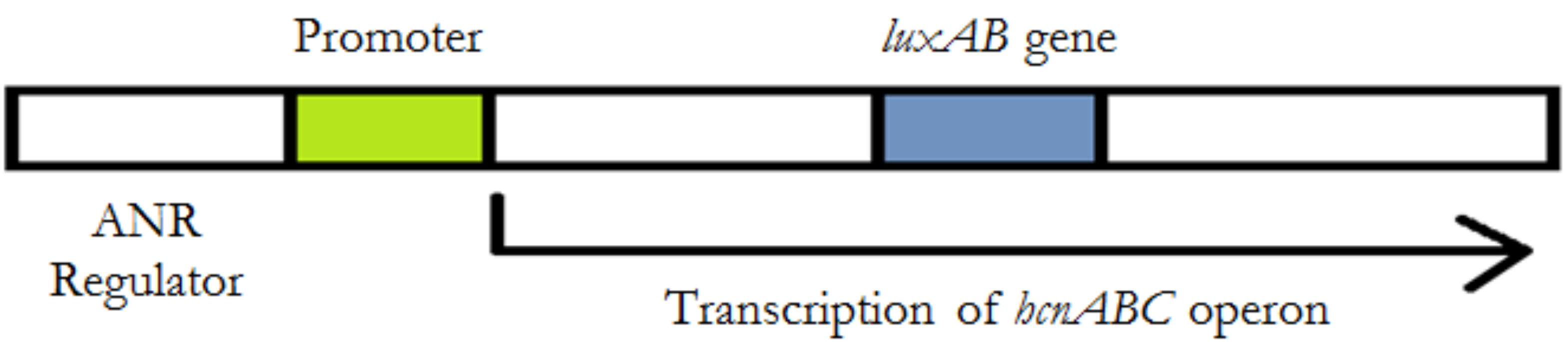
- Previous Research
  - Blumer and Haas (2000a)
    - Iron availability and minimal aeration activates ANR → increases HCN production in *P. fluorescens* CHA0
  - Pessi and Haas (2001)
    - Exponential bacterial growth activates GacA → increases HCN production in *P. aeruginosa* PAO1

Introduction

- Previous Research
  - Myrna Biswas (2010)
    - Cultures with iron and minimal aeration have greater HCN production in *P. putida*
      - Results were variable
  - Isaac Kim (2011)
    - Iron is necessary for HCN production in *P. putida*
      - Results not statistically significant

Introduction

- Current research
  - Varying iron and oxygen levels
  - Age of bacteria
  - Modified *hcnABC* operon

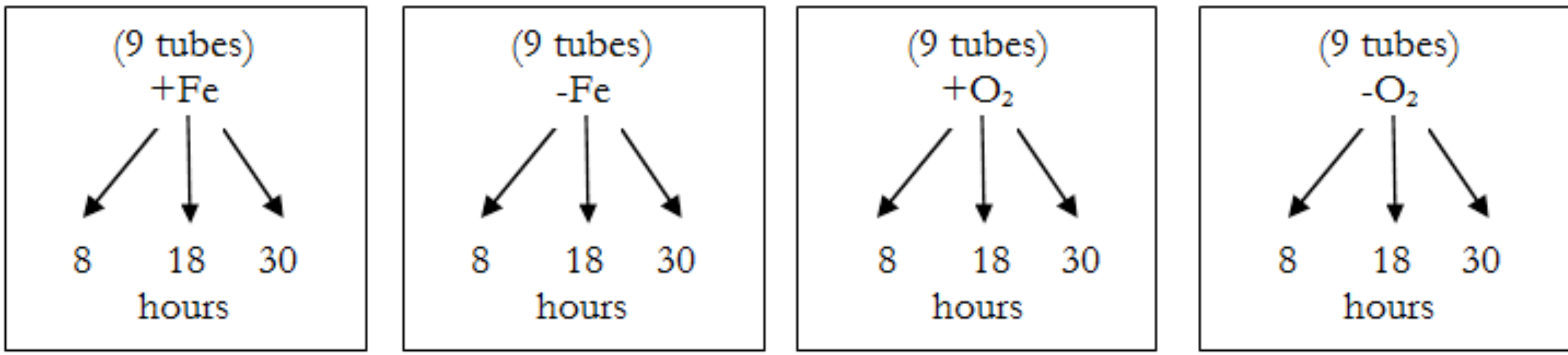


Methodology

- Culture conditions and enumeration
  - Strain of *P. putida* ATH2-1RI/9 with lux-modified *hcnABC* operon
  - Shaking water bath for 24 hours
  - Centrifugation and resuspension in 0.5 mL water → inoculum

Methodology

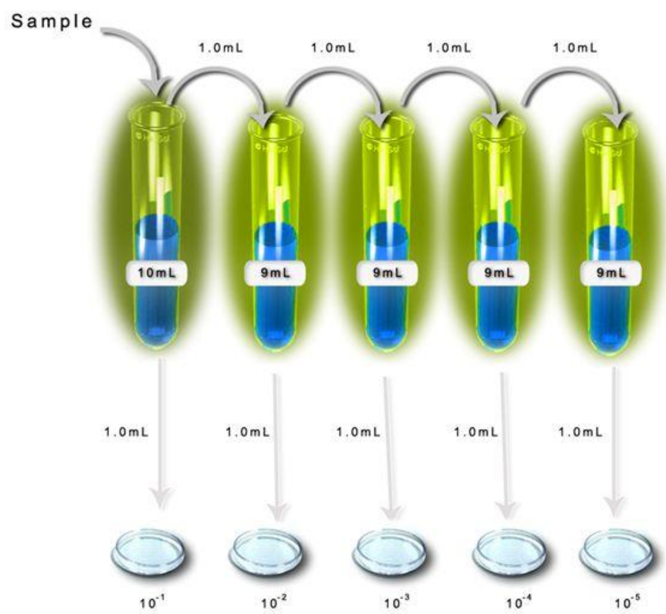
- Culture conditions and enumeration (continued)
  - Set-up of cultures



- 10 mL MMC media per test tube
- 10 μL of inoculum per test tube
- Iron solution: 0.0003244 g $FeCl_3$/100 mL MMC media

Methodology

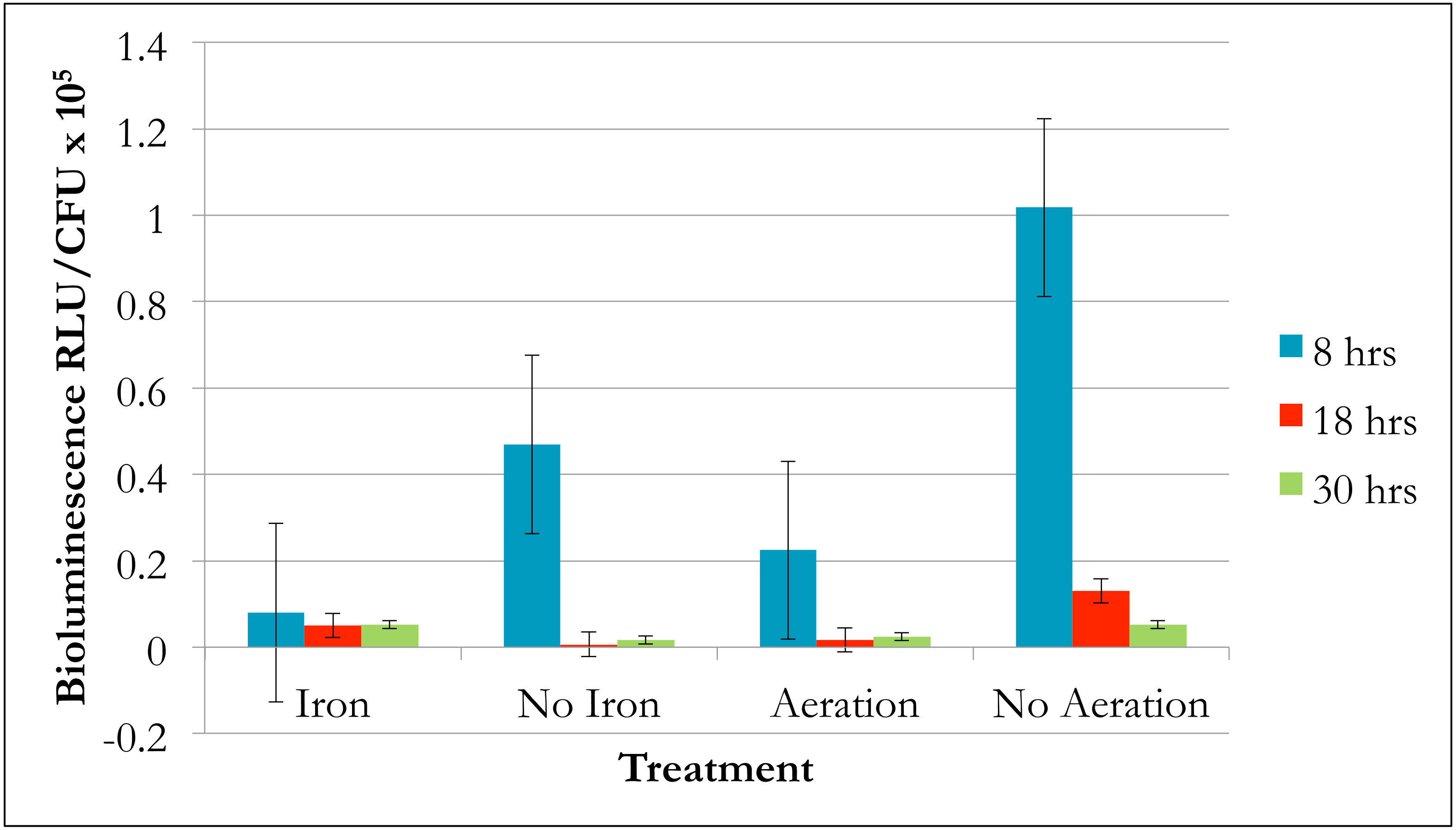
- Culture conditions and enumeration (continued)
  - Bacterial populations determined by dilutions and spread plating



Methodology

- Luminometry
  - 0.5 mL of culture added to luminometer tube
  - 10 μL added of 1% decyl alcohol in 100% alcohol
  - Spreadsheet Interface Software – 2 minutes
- Spectrophotometry
  - Absorbance at 600 nm

Results

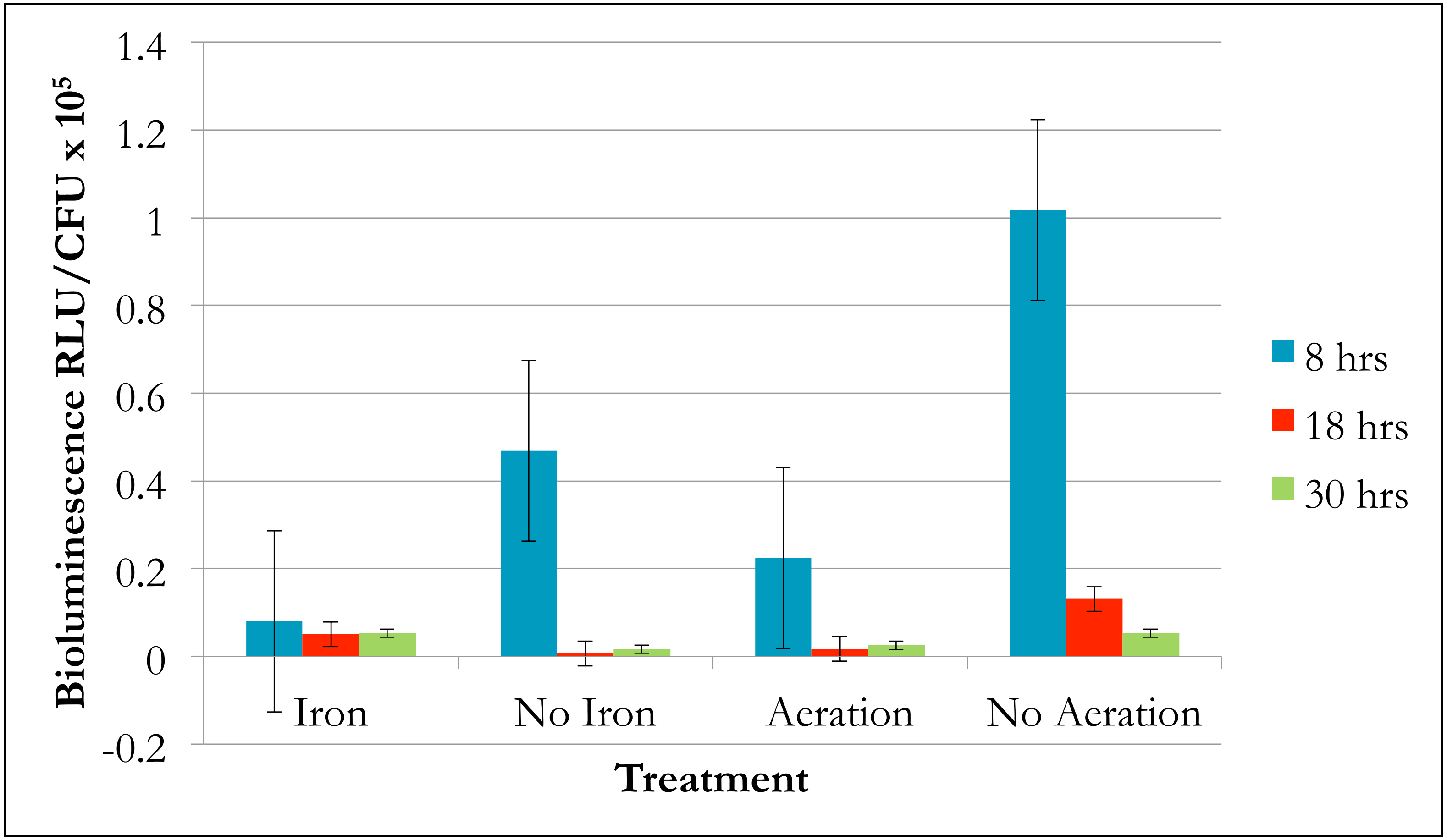


Not statistically significant: ($F_{(3,2)} = 0.561$, $p = 0.05$)

Discussion

- Prediction: Presence of iron increases HCN production
  - Laville et al (1998): Iron availability → Activates ANR → Turns on transcription of *hcnABC* operon → Increased levels of HCN production
    - *P. fluorescens* CHA0
  - Results compared to previous findings
    - Myrna Biswas (2010)

Results

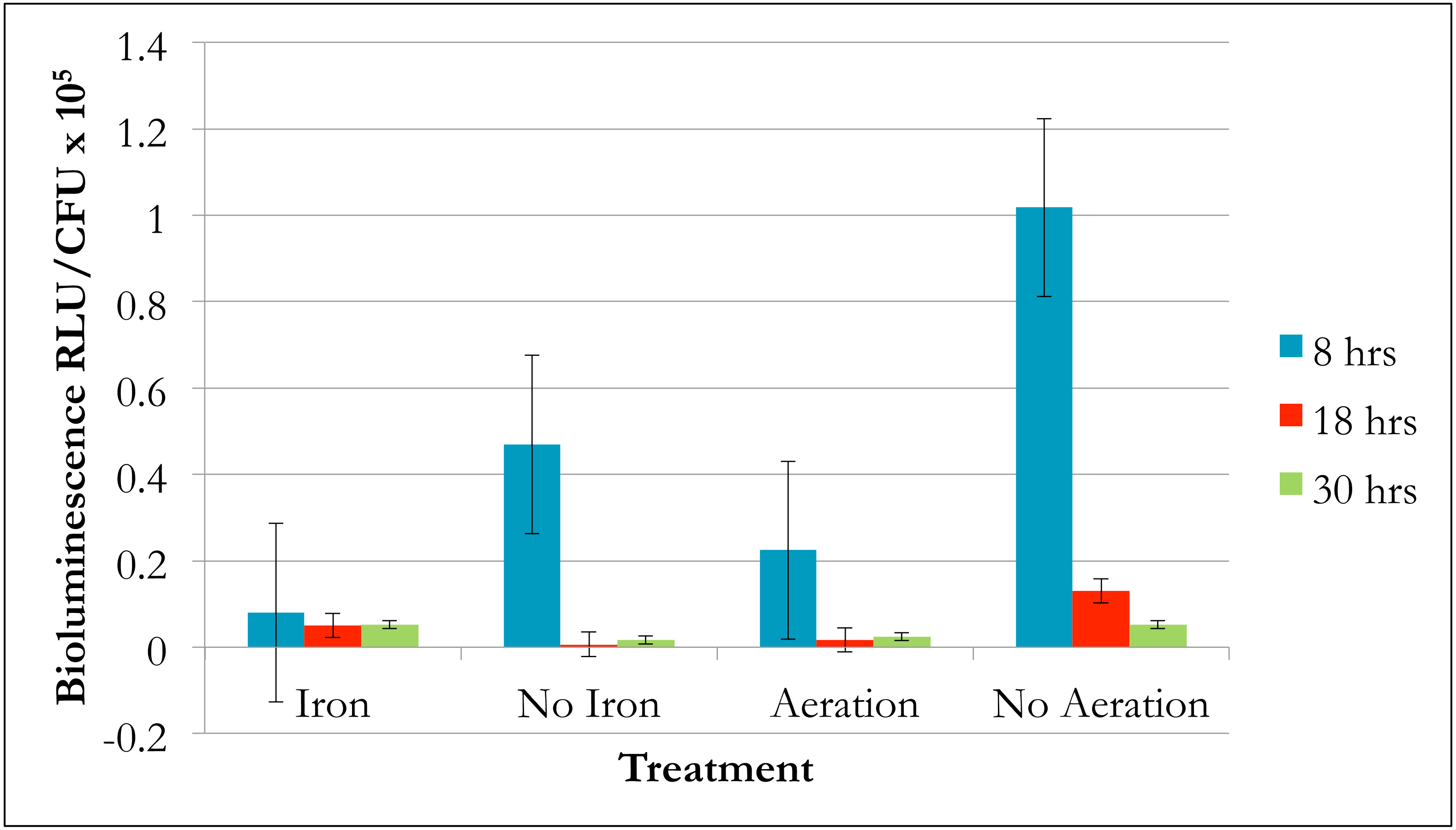


Not statistically significant: ($F_{(3,2)}$ = 0.561, p = 0.05)

Discussion

- Prediction: Low levels of oxygen, high levels of iron increase HCN production
  - Blumer and Haas (2000a): Low oxygen levels, high levels of iron → Activates ANR → Turns on transcription of *hcnABC* operon → Increased levels of HCN production
    - *P. fluorescens* CHA0
  - Results compared to previous findings
    - Myrna Biswas (2010)

Results

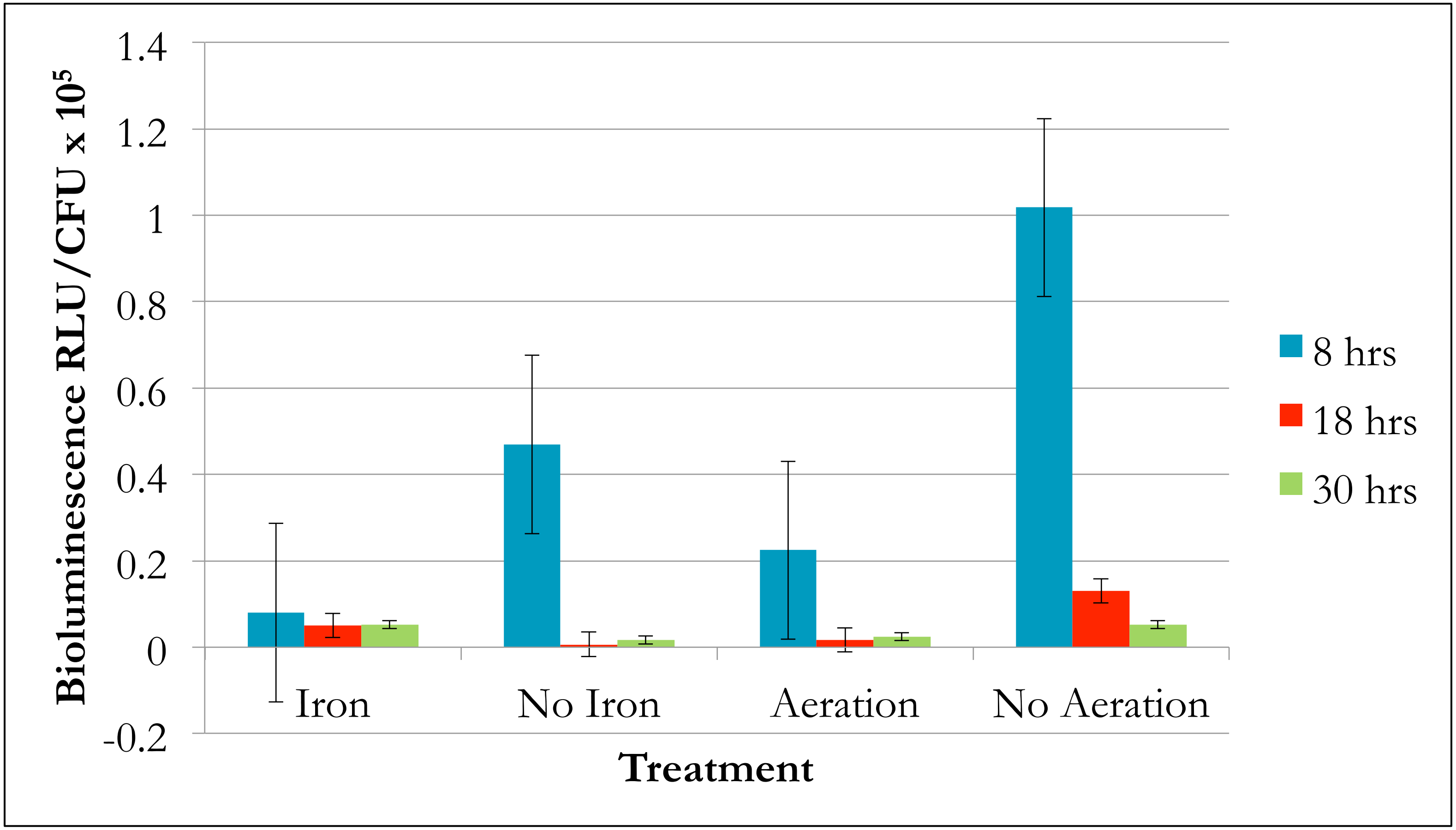


Not statistically significant: ($F_{(3,2)} = 0.561$, $p = 0.05$)

Discussion

- Prediction: Exponential cell growth increases HCN production
  - Pessi and Haas (2001): High bacterial populations → Activates GacA → Turns on transcription of *hcnABC* operon → Increased levels of HCN production
    - *P. aeruginosa* PAO1
- Results compared to previous findings
  - Pessi and Haas (2000)

Results



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Further Study

- Variability in iron and no iron treatments
  - Myrna Biswas (2010)
  - Current research
- At what population of bacteria does GacA turn off transcription and lead to a decrease in HCN production?

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Acknowledgments

- Dr. Robert Zdor – Advisor
- Andrews University Biology Department