Growing and Harvesting Nitrogenase Proteins from *Azotobacter Vinelandii*

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Abstract

To study the worth of the electronic interaction between constituent proteins FeP and MoFeP, in the enzyme Nitrogenase, I am growing and harvesting different types of bacteria: A wild type bacteria, *Azotobacter vinelandii* which is naturally found and can make Nitrogenase easily, the mutant βK400E, which changes a positive ion on the MoFeP to become negative (thus causing a weaker connection between the FeP and the MoFeP), and finally L127Δ, which takes away a part of the FeP, causing the FeP to become stuck in place to the MoFeP.

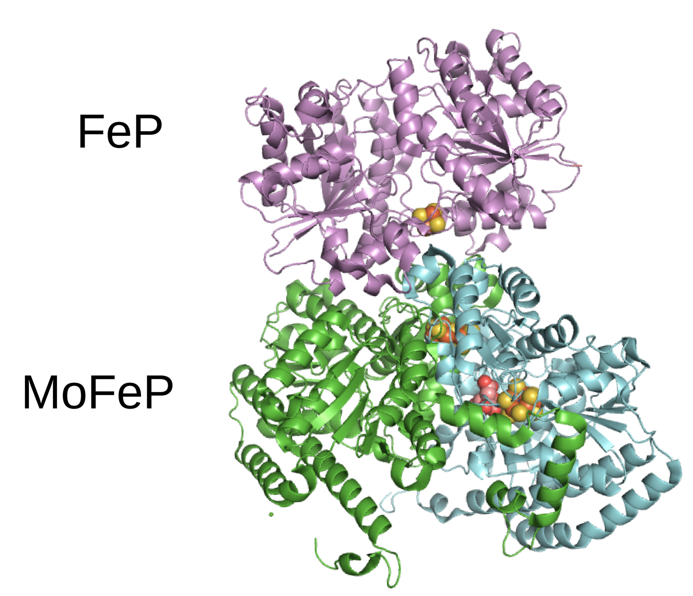
Background

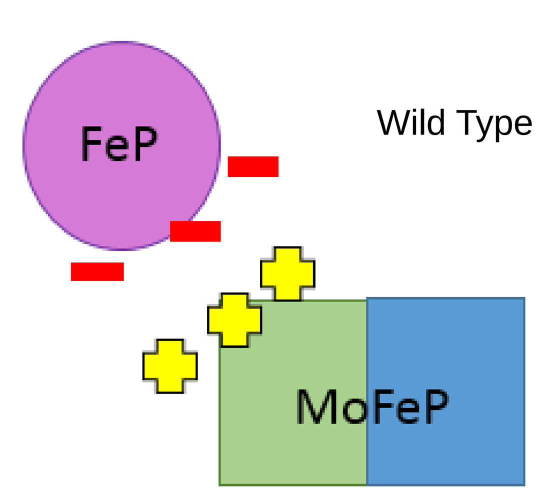
- Ammonia
  - Needed by countless organisms
  - Pharmaceuticals
  - One of the highest-produced chemicals in the world
  - Can be made by Nitrogen fixation (slow):

$$N_2 + 8\,e^- + 16\,ATP + 8\,H^+ \longrightarrow 2\,NH_3 + 16\,ADP + 16\,Pi + H_2$$

- Nitrogenase
  - Only enzyme that can turn dinitrogen from the atmosphere into ammonia
  - Within the enzyme itself, the Fe protein (FeP) transfers 1 electron to the connected MoFe protein (MoFeP) with each association

Growing the Wild Type

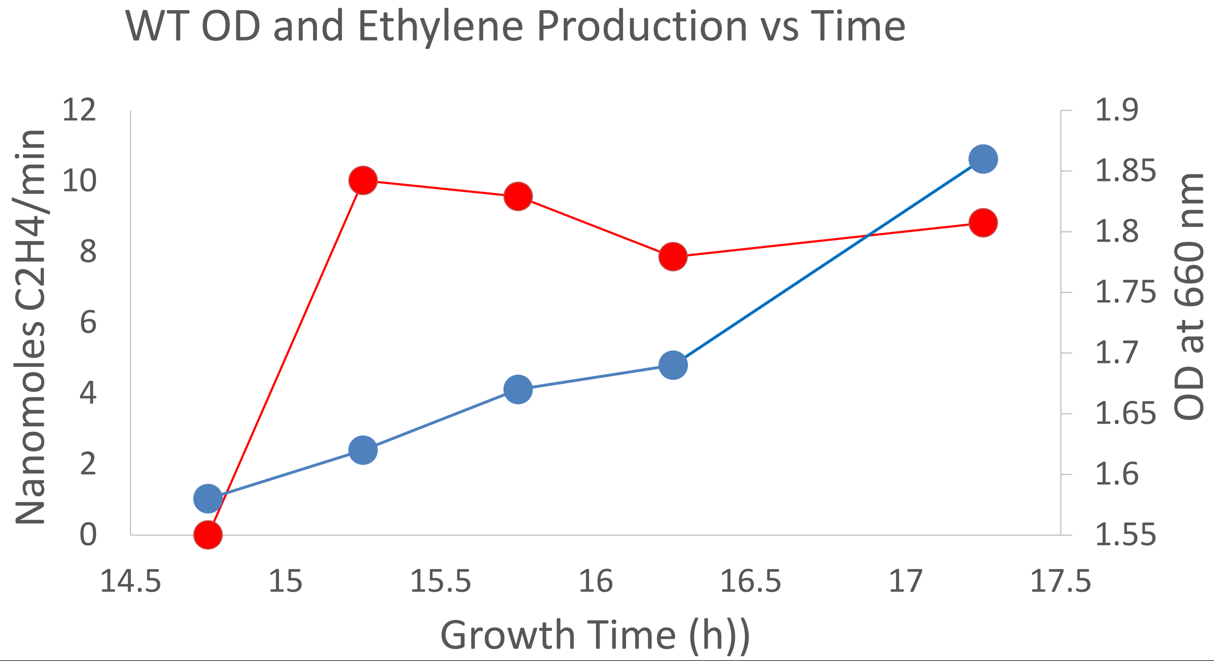


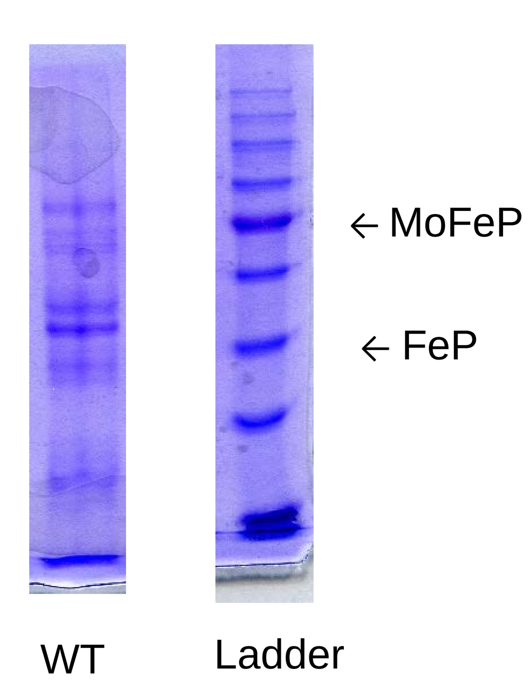


- Wild Type: [N+]
- Grown in fermenter
- Measure amount of ethylene to know when to harvest bacteria

$$C_2H_2 + 2e^- + 2H^+ \longrightarrow C_2H_4$$

WT OD and Ethylene Production vs Time



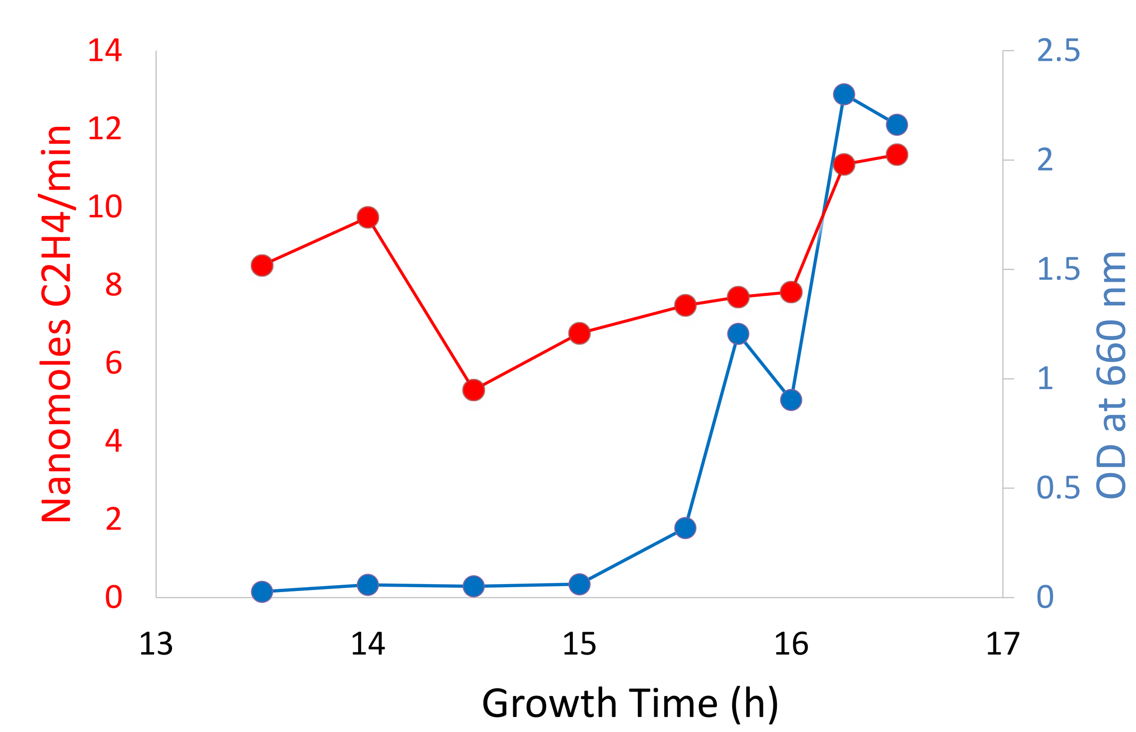


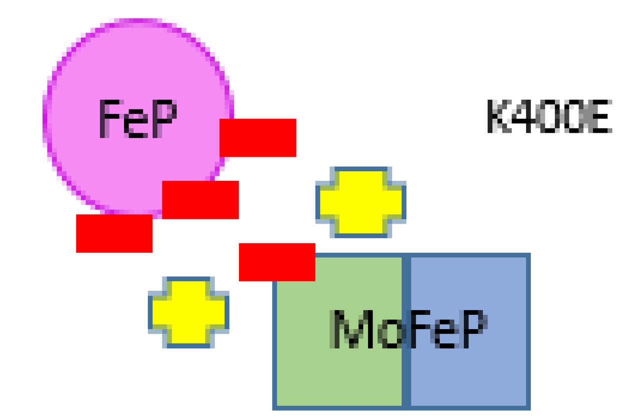
Growing the Mutants

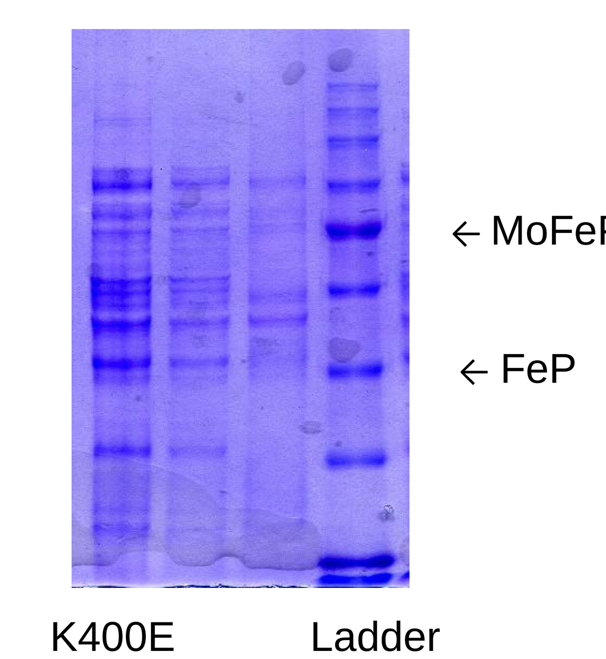
βK400E: [N "slow"]

- Activity of mutant is not as efficient as WT
- Could cause cells to grow slower

K400E OD and Ethylene Production vs Time



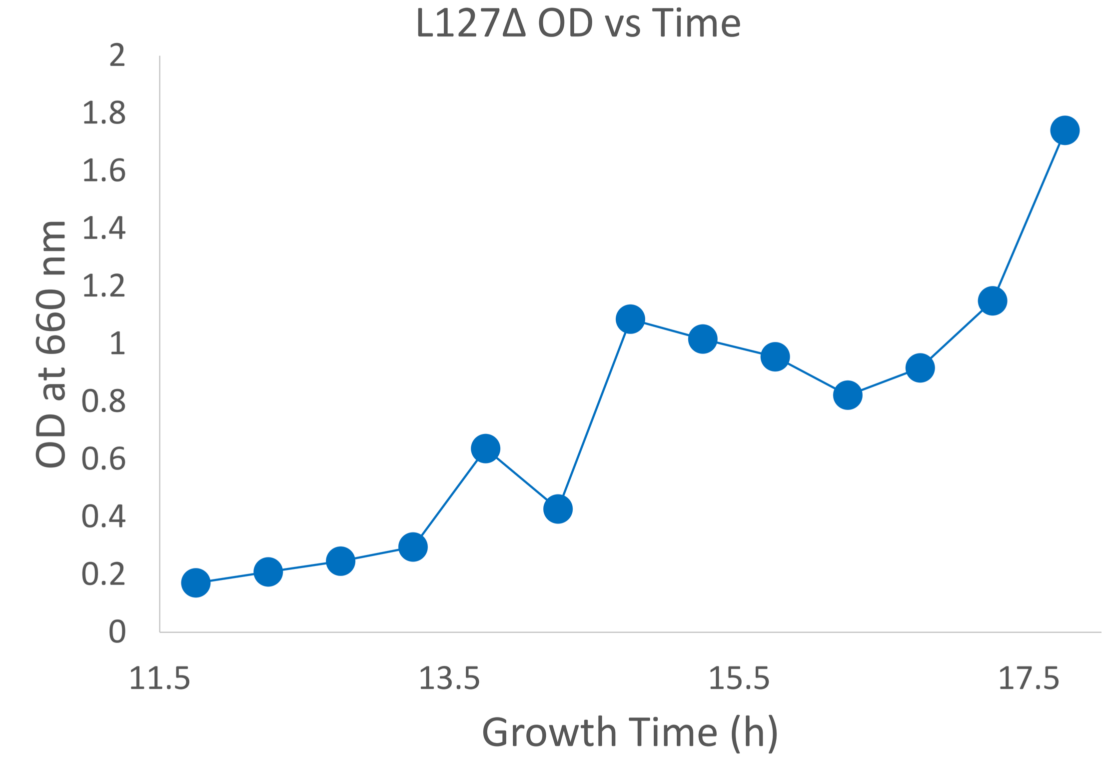


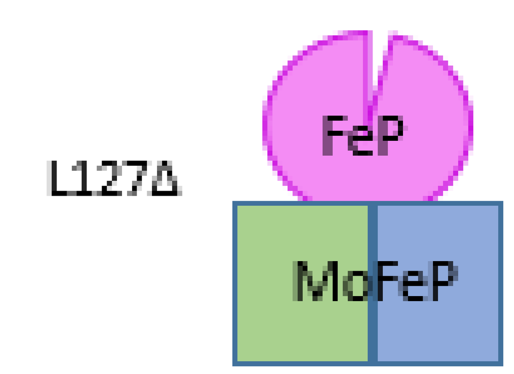


L127Δ: [N -]:

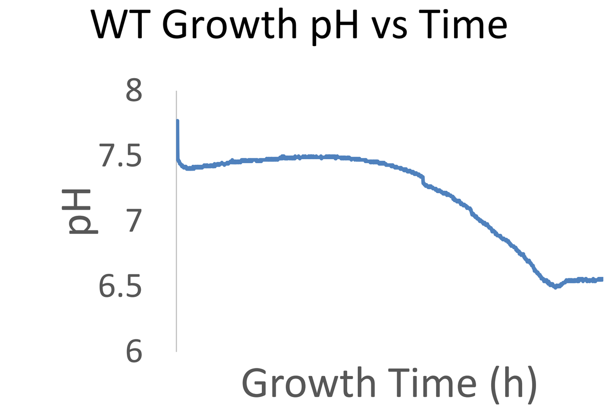
- FeP stuck in place on MoFeP
- We hypothesize that the cell will not be able to produce ammonium from the nonviable Nitrogenase

L127Δ OD vs Time





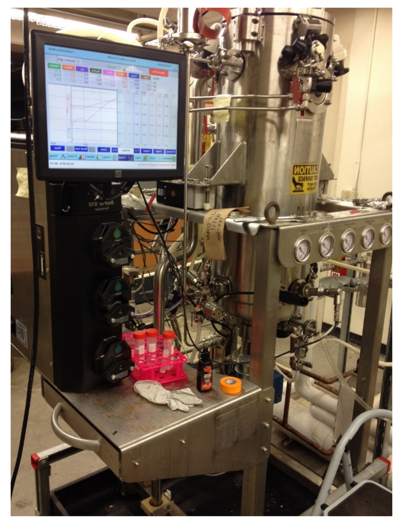
WT Growth pH vs Time



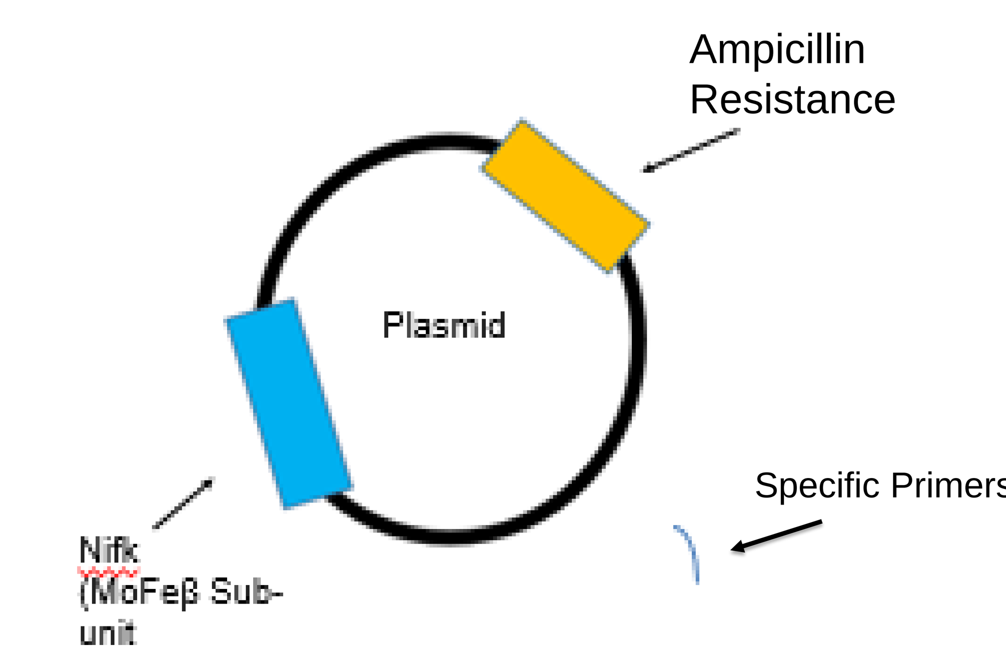
(Correlates to L127 Δ growth)

Growth Conditions in Fermenter

Burke's medium (BM) :
0.2% sucrose
0.9 mM $CaCl_2$
1.67 mM $MgSO_4$
0.035 mM $FeSO_4$
0.002 mM $Na_2Mo_2O_4$
10 mM Na_3PO_4 (pH 7.4)
10 mM NH_4Cl



Future Directions



- Cracking cells open to find proteins
- Purifying proteins
- Testing:
  - Changing variables: pH, salt concentration, etc.
- More Mutants

Conclusions

By growing and harvesting different types of cells such as the Wild Type, βK400E mutant, and L127Δ mutant, I learned about the different effects each strain has on the proteins produced. I saw that the K400E mutant produced about the same amount of Ethylene, but at a quicker rate than the Wild Type. The L127Δ's OD seemingly took longer to grow and gave us a smaller amount of protein because the cells make defective Nitrogenase that does not allow them to produce ammonia. We tried to harvest the L127Δ at the point when the cells were still making Nitrogenase and had the MoFeP and FeP that we want to crack out of the cells.

Acknowledgments

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