Influence of Iron Concentration on the Expression of the Ferripyoverdine Receptor Gene FpvAIIb of *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa is an environmental bacterium which opportunistically infects humans. Commonly residing in iron-depleted environments, *P. aeruginosa* uses iron-chelating molecules called siderophores to scavenge iron from its environment. The most prevalent siderophore used by *P. aeruginosa* is known as pyoverdine. After the cell synthesizes and secretes pyoverdine, the molecule binds with high affinity to iron in the environment. The iron-pyoverdine (ferripyoverdine) complex is brought into the cell upon binding to the ferripyoverdine receptor FpvA, and the iron is used to carry out critical cell functions.

Previous studies in our lab have used pyocin S3, which is a protein toxin that enters target cells through a sub-type of the ferripyoverdine receptor called FpvAIIb in *P. aeruginosa* ATCC 27853. These studies have shown that pyocin S3 is able to kill cells independently of the iron concentration in the medium, suggesting that the FpvAIIb receptor is expressed equally in different iron concentrations. This would be a novel expression pattern for FpvA, which is currently known to be inversely regulated with iron availability in the strain PAO1. The aim of this research study is to determine whether *P. aeruginosa* ATCC 27853 expresses the FpvAIIb gene in an iron-independent manner.

Methods

Quantitative real-time polymerase chain reaction (qPCR) was used to study the expression of FpvAIIb. This technique amplifies the gene of interest (FpvA) from a pool of DNA in real time to determine the original concentration of genetic transcripts which were present in the cell. FpvAIIb expression in *P. aeruginosa* ATCC 27853 was compared to FpvA expression in the strain PAO1, which is known to be iron-regulated.

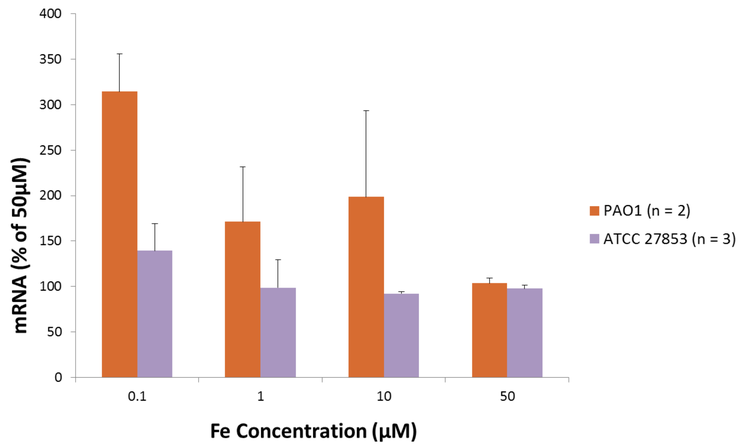
Each strain was grown in a casamino acid (CAA) based growth medium containing different amounts of $FeCl_3$. These concentrations ranged from iron-depleted (0.1µM $FeCl_3$) to iron-replete (50µM $FeCl_3$) conditions. After overnight growth, RNA was extracted from each sample. The extracted RNA pool, which contains mRNA from all genes being expressed in the cell under the given conditions, was converted to complementary DNA (cDNA) using a reverse transcription reaction. This DNA was then used for qPCR analysis. The entire protocol was performed at 37°C and 30°C to determine if expression is dependent on growth temperature.

Results

FpvAIIb is regulated independently of iron availability.

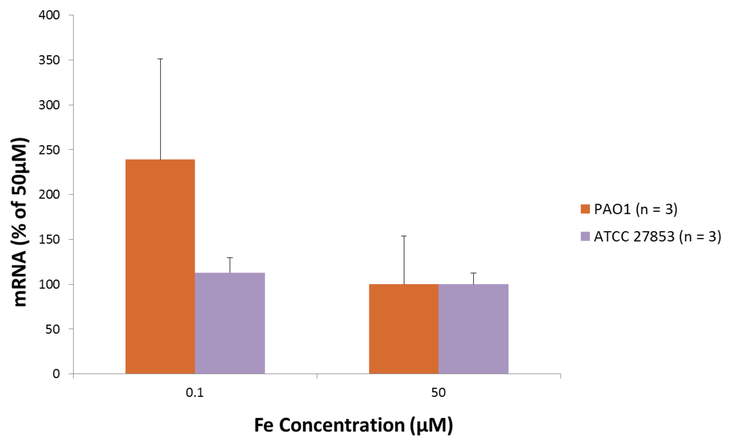
mRNA concentrations of the FpvAIIb gene were found in nearly equal quantities under all iron conditions (Figure 1). This contrasts the iron-regulated system used in the strain PAO1. The regulation of FpvA in PAO1 is currently the only characterized model of regulation of the pyoverdine system.

Figure 1: Pyoverdine Receptor Expression at 37°C



Due to previously observed variations in pyocin S3 killing activity at different temperatures, the qPCR experiment was repeated at 30°C. The same overall pattern of regulation was observed at 30°C as 37°C for both strains (Figure 2).

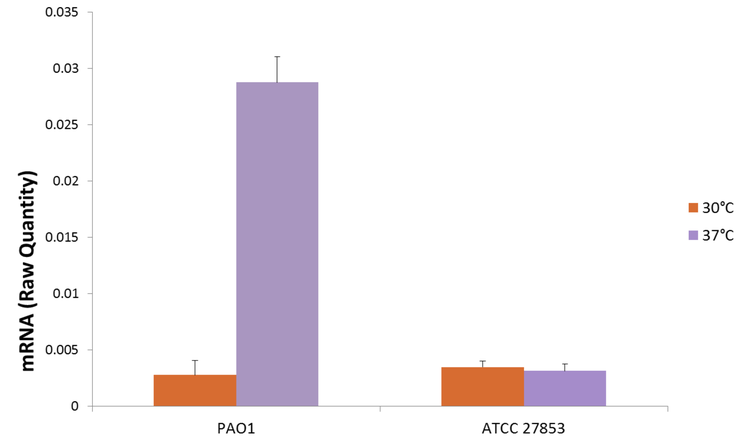
Figure 2: Pyoverdine Receptor Expression at 30°C



FpvA Expression is Dependent on Growth Temperature in strain PAO1

A direct comparison of FpvA transcripts at different temperatures reveals an interesting result: the pyoverdine receptor does not undergo temperature-dependent regulation in ATCC 27853,as expected, however a large difference in expression was observed in the strain PAO1.

Figure 3: Pyovedine Receptor Expression at 30°C and 37°C (0.1µM Fe)



Conclusions

This experiment was able to support the hypothesis that the pyoverdine receptor FpvAIIb in *P. aeruginosa* ATCC 27853 is regulated in an iron-independent manner. In addition, this study also provides evidence that the observed variation in pyocin S3 activity at different temperatures is not due to differential expression of FpvAIIb. Future studies should confirm the presence of the FpvAIIb protein on the cell surface under different iron conditions. In addition, the explanation for temperature-dependent pyocin S3 activity remains elusive and would be a good candidate for future experiments.

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References

1. **Baysse C, Meyer JM, Plesiat P, Geoffroy V, Michel-Briand Y, Cornelis P.** 1999. Uptake of Pyocin S3 Occurs through the Outer Membrane Ferripyoverdine Type II Receptor of *Pseudomonas aeruginosa*. Journal of Bacteriology. **181**(12):3849-3851.
2. **Meyer JM, Stintzi A, De Vos D, Cornelis P, Tappe R, Taraz K, Budzikiewicz H.** 1997. Use of siderophores to type pseudomonads: the three *Pseudomonas aeruginosa* pyoverdine systems. *Microbiology*. **143**: 35-43.