EFFECT OF IRON IN BACTERIAL GROWTH & FBP PRODUCTION

Student(s)

Gizem Çile
İlknur Şafak Demirel

Faculty Member(s)

Gökşin Liu
Zehra Sayers

Sabancı Üniversitesi

PURE
PROGRAM FOR UNDERGRADUATE RESEARCH

ABSTRACT

FBP is an iron binding protein in gram negative bacteria that has the molecular weight of 37 kDa[1]. It sequesters iron from transferrin which is an iron binding protein in humans. If the iron-binding of FBP is prevented, the bacteria would die as it can not take iron from transferrin[2] Since an alternative drug to antibiotics can be found, further investigations of FBP structure is needed. We focused on the optimization of iron concentration to synthesize more recombinant FBP in bacteria to be used in structural studies. After the optimization of iron concentration, we have made the purification of our protein of interest.

OBJECTIVES

As *E.Coli* do not normally express FBP, the plasmid pET28a (+) containing Kanamycin resistance and FBP gene was inserted into BL21 strain of *E.Coli* which is ideal for protein expression. In order to investigate the structure of FBP, the expression of FBP in BL21 should be increased via optimizations. Therefore in this study, we worked on the concentrations of iron for optimizing expression and performed purification to obtain the expressed FBP and for its quality control .

METHODS & RESULTS: Expression

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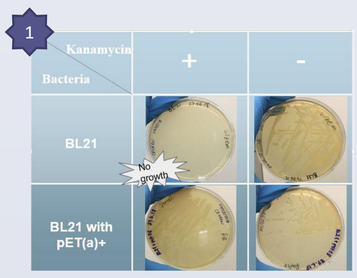

Table 1: Four different plates were prepared with and without pET28a(+) in KAN(+) and KAN(-) environments.

1-Plate Preparation

Bacteria were seeded as seen in Table 1.

2-Growth Culture

A chosen colony from Kanamycin environment was added to four flasks in 100 ml LB medium and growth at 37 °C was monitored at different iron concentrations.

3-FBP Expression via Induction Culture

When the OD_{600} value reaches to 0.65, IPTG was added into 100 ml of bacterial culture in four flasks with different iron concentration to induce FBP gene expression.

4-Data Analysis

Optical densities at 600 nm were recorded every hour and graphs were plotted to check the growth rates of the populations.

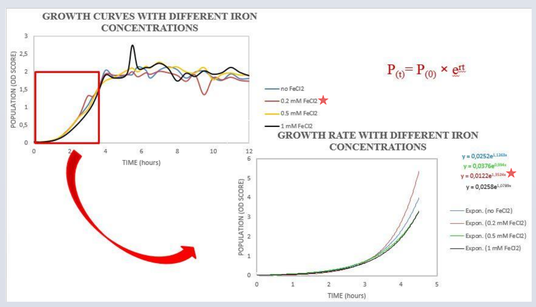

Figure 1: Growth and rate curves of bacteria with different iron concentrations. The above formula was used to calculate the rate of growth.

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Bacterial Growth & Induction Cultures				
Purpose \ C_{Fe}	No $FeCl_2$	0,2 mM $FeCl_2$	0,5 mM $FeCl_2$	1 mM $FeCl_2$
Bacterial Growth	•LB •KAN •Culture	•LB •KAN •Culture •Fe	•LB •KAN •Culture •Fe	•LB •KAN •Culture •Fe
	12 hours of OD measurements	12 h OD measurements	12 h OD measurements	12 h OD measurements
Induction	At OD_{600} =0,65			
	0,7 mM IPTG	0,7 mM IPTG	0,7 mM IPTG	0,7 mM IPTG
	5 hours of expression	5 h expression	5 h expression	5 h expression

Table 2: Bacterial growth and induction cultures with four different iron concentrations.

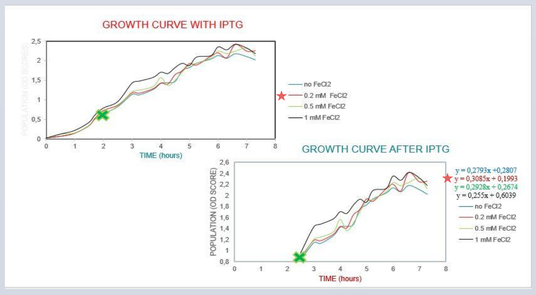

Figure 2: Growth curves of bacteria with IPTG addition

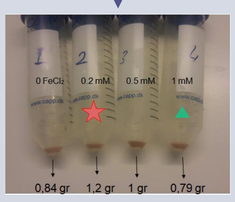

Figure 3: Different yields with different iron concentrations. When FBP is bound to iron source, the color turns to pink.

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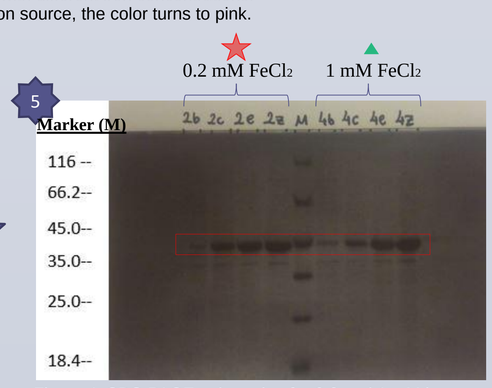

Figure 4: SDS-PAGE gel running for 0.2 and 1 mM iron concentrations. t= time after IPTG addition (expression). Samples b,c,e,z: t=0,1,3,5 respectively.

5-12% SDS-PAGE Analysis

FBP gene expression of 0.2 mM and 1 mM iron concentrations was checked.

METHODS & RESULTS: Purification

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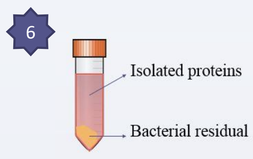

Figure 5: Bacteria were lysed with BugBuster protocol

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Figure 6[2]: FBP was purified by using Nickel affinity column. Elution Buffer has Imidazole in it that binds to Nickel to elute FBP.

6-Lysis with BugBuster Protocol

The pellet was lysed and centrifuged and isolated proteins containing supernatant was taken.

7-FBP Purification

Supernatant were poured into Ni-affinity column, Washing Buffer and Elution Buffer were added respectively. In each steps, samples were taken.

8-Dialysis

PBS Buffer was used.

9-SDS-PAGE Analysis

The samples were runned in SDS-PAGE gel electrophoresis.

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Figure 7: The remaining Imidazole was removed by dialysis with PBS Buffer.

Figure 8: Purified FBP after dialysis.

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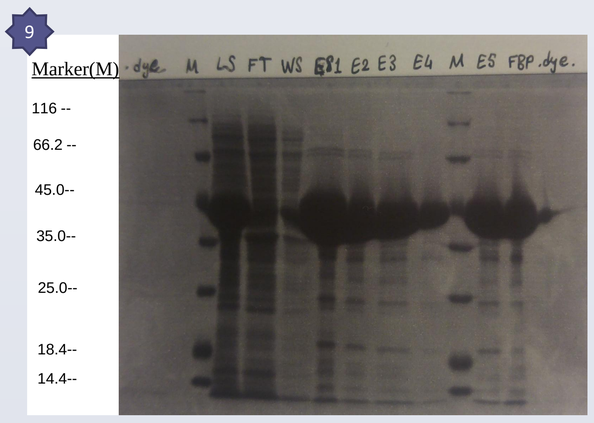

Figure 9: SDS-PAGE gel running.
LS: Supernatant after lysis, FT: Flow through, WS:Sample after washing, ES(1-5): Samples after elution, FBP: Sample after dialysis

After dialysis, the concentration of FBP was measured as 2.54 mg/ml by NanoDrop.

CONCLUSION

As four different $FeCl_2$ concentrations of 0 mM, 0.2 mM, 0.5 mM and 1 mM were examined, the samples with 0.2 mM of iron concentration has;

- The highest yield with 1.2 gr,
- The highest growth rate obtained from analysis and modelling of OD_{600} data,
- Thicker bands at 37 kDa in SDS-PAGE gel screening which indicates higher FBP expression.

Therefore, we concluded that the best iron concentration to be used in further experiments is 0.2 mM for both the optimum bacterial growth and FBP production.

REFERENCES

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2. Krewulak, K. D., & Vogel, H. J. (2007, August 19). Structural biology of bacterial iron uptake. Retrieved from https://www.sciencedirect.com/science/article/pii/S0005273607002738
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CONTACT US:

Gizem Çile — gizemcile@gmail.com
İlknur Şafak Demirel — ishefak@gmail.com