Expression and Characterization of a Metalloprotein in Bacteroides Fragilis

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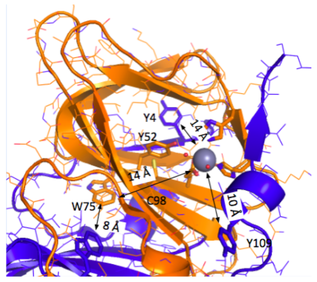
BF4112:

Orphan protein BF4112 (from B. fragilis) was identified from the protein data bank as a possible Cys-Tyr containing protein. BF4112 is a metalloprotein and contains a cysteine residue (Cys98) in close proximity to a tyrosine residue (Tyr52), as well as a nearby metal binding site.

Figure 1: Crystal structure of apoBF4112.

Figure 2: apoBF4112 active site. Distances between key residues shown. Cross-link forms between Y52 and C98. Sphere represents metal ion.



Cys-Tyr:

Cysteine-Tyrosine modifications are a specific class of tyrosine PTM and remain relatively uncharacterized. We suggest that Cys-Tyr modifications may be more abundant than previously thought. The relevant thioether functional group forms at the 3' carbon of a tyrosine residue. Importantly, the few Cys-Tyr PTMs that have been identified are redox-active cofactors.

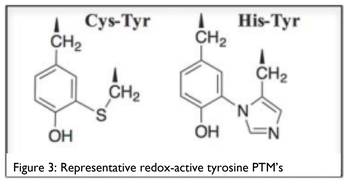
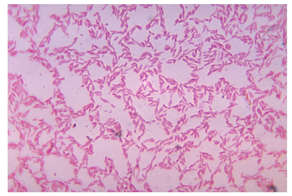


Figure 3: Representative redox-active tyrosine PTM's

Bacteroides fragilis:

B.fragilis is an anaerobic gut bacteria that is the most common isolate from pathogenic gastrointestinal infection. While B. fragilis is an anaerobe, its aerotolerance is well documented and likely contributes to its pathogenicity. While we have expressed BF4112 in E. coli, we sought to express BF4112 in B. fragilis to characterize the protein in its native environment. Characterization includes: whether Cys-Tyr forms, the native metal ion, and the function of the protein.

Figure 3: Bacteroides fragilis
Image:CDC/Dr. V.R. Dowell, Jr.



Methods

The following procedure was developed and optimized to express BF4112 in Bacteroides fragilis:

- Electrocompetent B. Frag cells were prepared and subsequently electroporated with His-BF4112 or pER 153 plasmid DNA.
- Transformants grew on TSA plates supplemented with hemin, cysteine, and erythromycin for 2 days.
- Single colonies were grown to 1 liter (or 25mL) anaerobically. Maltose was added to induce expression.
- BF4112 1L cultures were pelleted and suspended in Tris buffer before lysis using French Press.
- Cell lysate was purified using affinity chromatography
- Protein was concentrated, ran on SDS PAGE, and analyzed for iron content using the iron-binding agent ferrozine.
- pER 153 cultures (25mL) were lysed using sonication and analyzed using a fluorimeter.

Expression

The flavin-mononucleotide-fluorescent-based protein (FbFP) encoded by plasmid pER 153 (with maltose inducible promoter) was used to validate and optimize expression. pER 153 was subcloned to express His-BF4112. Experiments tested the effects of maltose addition and optimized the time period and amount of maltose for maximal protein expression in B. fragilis.

Figure 4: FbFP expression +/-maltose.

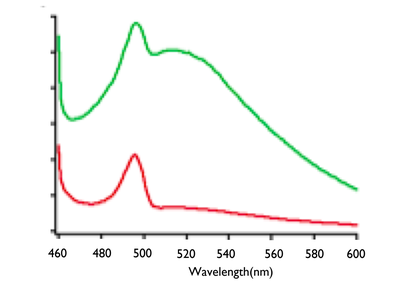
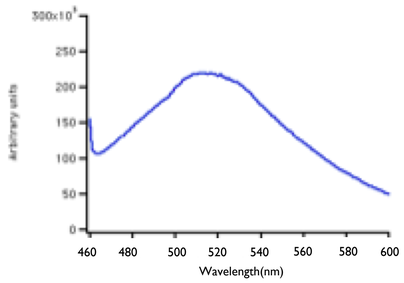


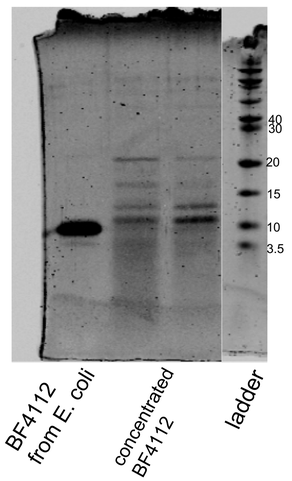
Figure 5: Fluorescence difference between two lines in Figure 5.



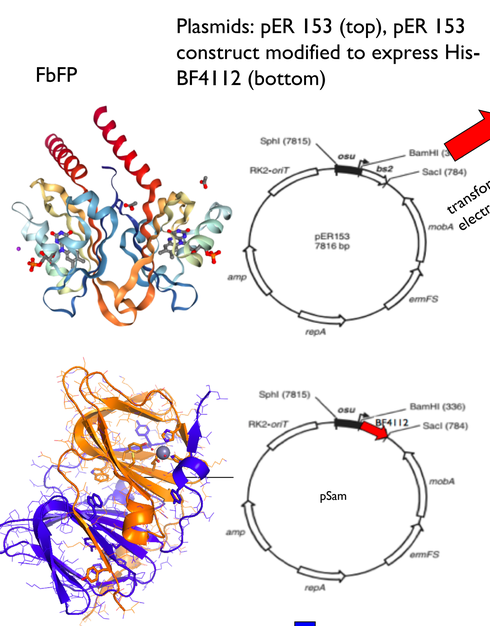
- Addition of maltose at .5% shows significant fluorescence increases with a maximum at 513nm
- Expression is relatively constant from 15-30 hours.

Time (h)	Fluorescence
15	1.45E+06
18	1.35E+06
21	1.05E+06
24	1.36E+06
27	1.19E+06
30	1.33E+06

Figure 6: SDS PAGE gel showing maltose induced His-BF4112 expression, isolated from Bacteroides fragilis.



His-BF4112 was isolated and concentrated using NanoSep concentrator tubes. SDS PAGE confirmed protein between 10 and 15kDa and in the range of apoBF4112. Double-banding indicates the slight electrophoretic shift caused by Cys-Tyr formation. This finding confirms the expression of BF4112 in Bacteroides fragilis and indicates the likely formation of Cys-Tyr in native protein.



Conclusions:

- BF4112 expression can be induced in Bacteroides fragilis using a plasmid construct
- Native BF4112 can be isolated and harvested
- Cys-Tyr likely forms in native BF4112

Future Directions:

- Confirmation of Cys-Tyr in native BF4112 by mass spectometry
- Further experiments into BF4112 function
  - Two hypotheses: Antioxidant or antibiotic-binding protein
  - Development of CRISPRi system to silence the gene for functional experiments
- Confirmation of native metal ion

Acknowledgements

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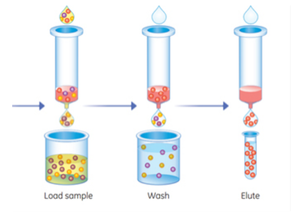
Load sample
Wash
Elute