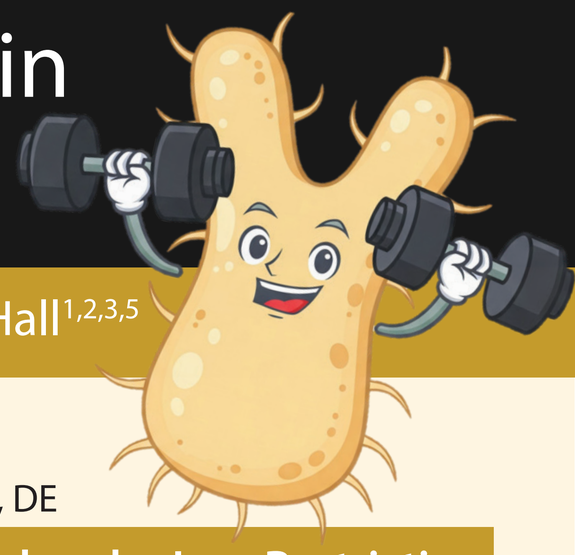
Pumping iron: exploring a novel iron transporter in the early life microbiota member ***Bifidobacterium***



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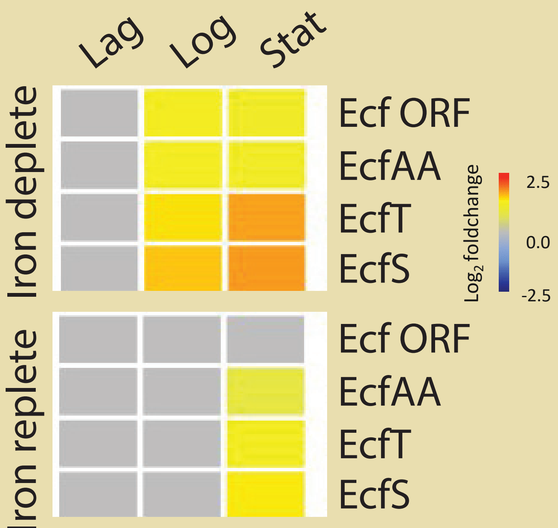
Introduction

- Iron is a essential for almost all life, but its bioavailability is limited, necessitating diverse iron aqusition strategies
- Very little is known about how beneficial bacteria, such as ***Bifidobacterium*** aquire iron within the gut.
- We identify and characterise a novel, iron uptake system in the early life microbiome member, ***Bifidobacterium longum***

ECF genes are upregulated during Iron Restriction



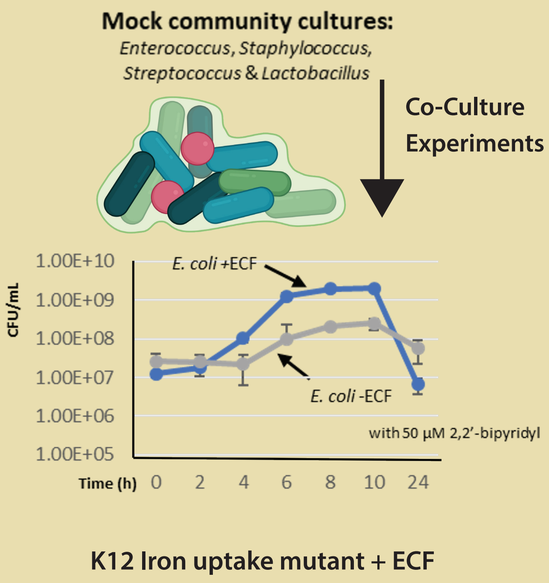
- ECF (Energy Coupling Factor) transport system is upregulated in low iron growth conditions
- ECF systems have not previously been linked to Iron uptake.

Fig2 - Differential expression of ECF genes in different growth phases of *B.longum* by RNA sequencing

ECF enhances growth in a gut microbial community

- *E.Coli* K12 iron uptake mutant was complemented with ECF system as heterologous host
- Grew in 5 species co-culture
- Presence of ECF resulted in a competitive advantage to K12, growing significantly better after 4 hours

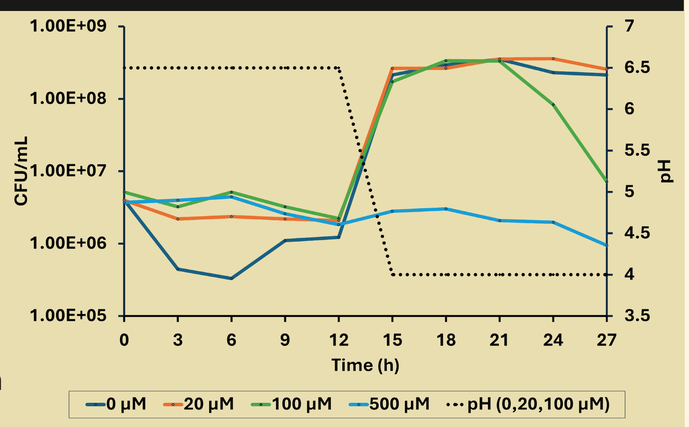
Fig4 - Growth of Iron uptake *E.coli* K12 mutant in a 5 species mixed community.



Bifidobacterium growth is reduced under Iron Restriction

- Growth of ***B.longum*** was severly affected by increased concentration of the Iron chelator 2,2'-bipyridyl

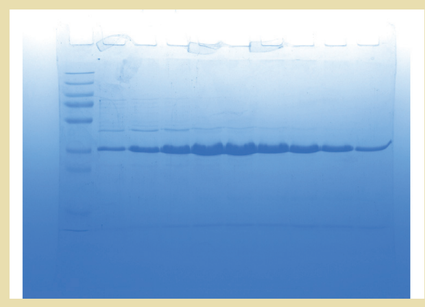
Fig1 - Growth of *Bifidobacterium longum* in the presence of varying concentrations of an iron chelator, 2,2'-bipyridyl

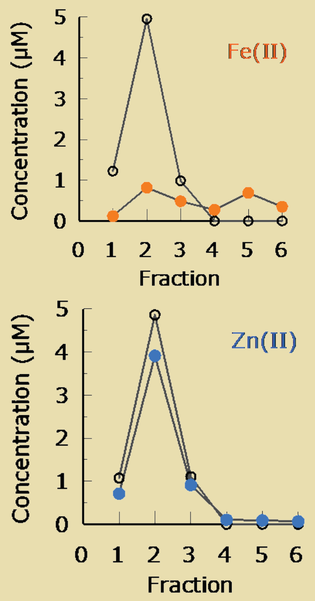


Purified *Bifidobacterium* ECF ORF specifically binds Iron

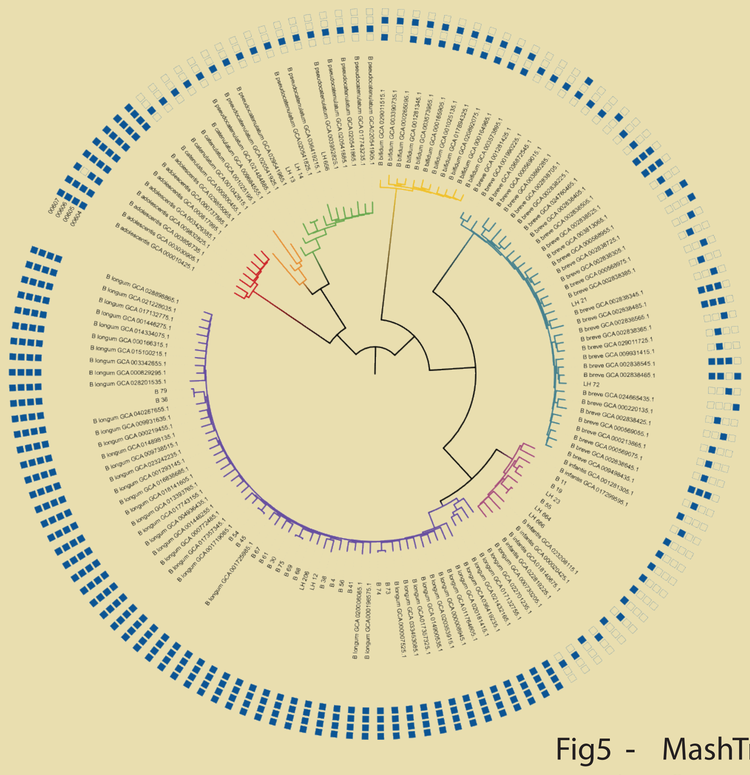
- Synthesised codon optimised gene for overexpression in *E. coli*
- Two step purification (without affinity tag)
  - ion exchange pH 6.5
  - SEC pH 7.5

Fig3 - Protein purification of LH277 ECF ORF and Iron and Zinc binding of purified protein





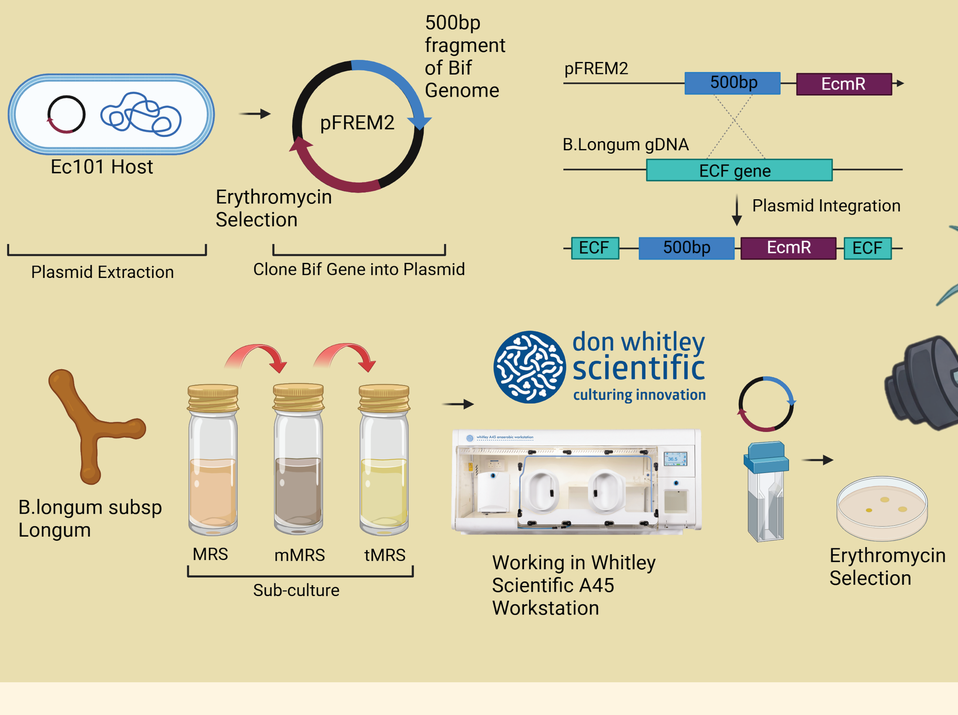
ECF is conserved within *Bifidobacterium longum* genomes



- Mashtree of publically available Bif genomes with Hall Lab isolates.
- Presence and absence of ECF genes identified by BLASTp (eValue = 1e-5)
- ECF genes present in other ***Bifidobacterium*** species

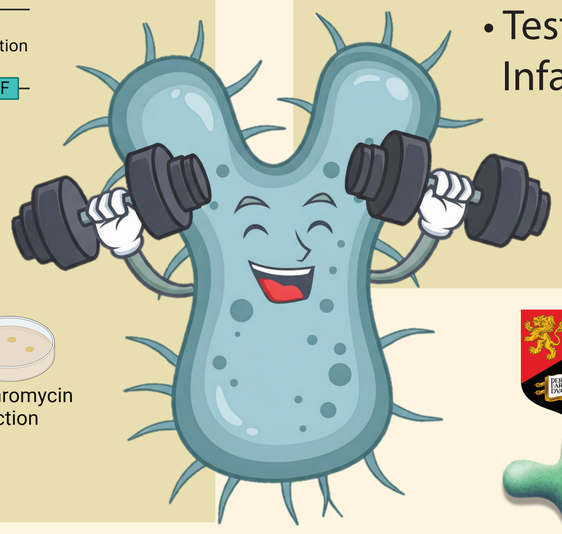
Fig5 - MashTree of human associated Bif species. Outer ring: S, T, AA, ORF : Inner Ring

Generation of ECF Mutants in *Bifidobacterium longum*



Future Directions

- ***Bifidobacterium*** ECF mutants will be phenotyped for their ability to grow under iron limited conditions
- Test ECF mutants within a synthetic community of Infant gut bacteria
- Information can be used to help inform Probiotic strategies or develop next generation probiotics



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