Structural homologs of Enterococcus faecalis LiaX-like genes in Bacillus subtilis provides new insights into the LiaFSR-mediated response associated with daptomycin resistance

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Background/Introduction

VRE (Vancomycin Resistant Enterococci)-associated infections are prevalent in healthcare settings and pose a significant therapeutic challenge. The lipopeptide antimicrobial daptomycin (DAP) is one of the last resort options to treat these organisms. Among VRE, mutations that confer DAP resistance (DAP-R) arise in proteins in the three-component signaling system LiaFSR, which is highly conserved in other Gram-positive pathogens. Similar to *Enterococcus faecalis* (*Efs*), LiaFSR in *Bacillus subtilis* (*Bsu*) is specifically activated by DAP and, thus, serves as a proxy for studying mechanisms of DAP-R. In *Bsu*, LiaF inhibits LiaR phosphorylation; however, the current model for DAP-R in *Efs* positions LiaF as an activator of LiaFSR directly regulated by the enterococcal-specific protein LiaX.

We postulate that the intimate association between LiaX and LiaF is crucial for DAP-R in enterococci. Using an *in-silico* approach, we compared LiaF from *Efs* and *Bsu* and searched for LiaX-like proteins in *Bsu*.

Methods

Protein predictions were determined by the RoseTTAFold algorithm (RoBetta, David Baker, UW). Structural comparisons were conducted using MatchMaker in UCSF Chimera. Paired sequence alignments were assembled through EMBOSS Needle.

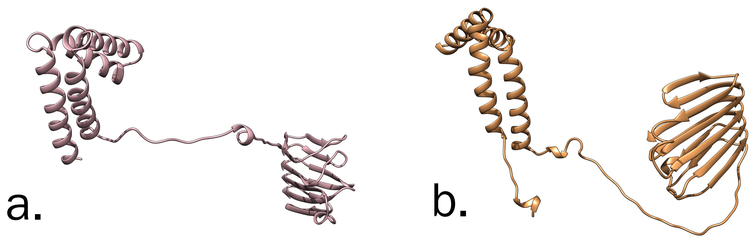

Figure 1. LiaF structural predictions from (a) *B. subtilis* and (b) *E. faecalis*. Each are organized similarly, with 4 putative N-terminal TM helices connected to a large C-terminal beta-sheet domain

References

Khan, A., et al., (2019) **PNAS**
Baek, M., et al. (2021) **Science**
Baker lab RobeTTa server online (http://robetta.bakerlab.org)
UCSF Chimera https://www.cgl.ucsf.edu/chimera/

Result

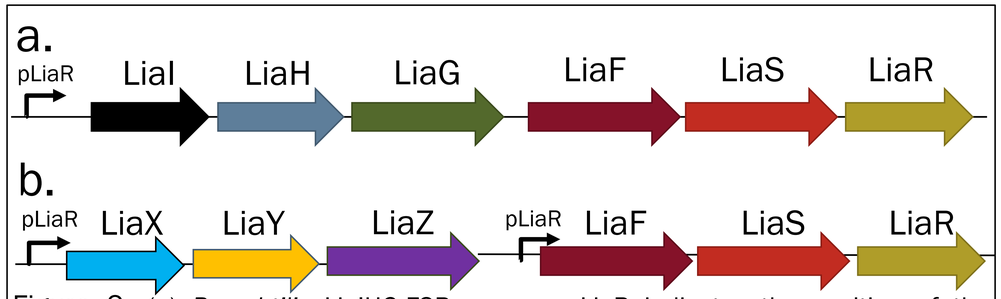

Figure 2. (a) *B. subtilis* LiaIHG-FSR operon. pLiaR indicates the position of the promoter for the polycistronic operon regulated by LiaR. (b) *E. faecalis* LiaFSR and LiaXYZ operons. pLiaR indicates promoters for each polycistronic operon regulated by LiaR. ORFs are colored similarly based on previously identified homology.

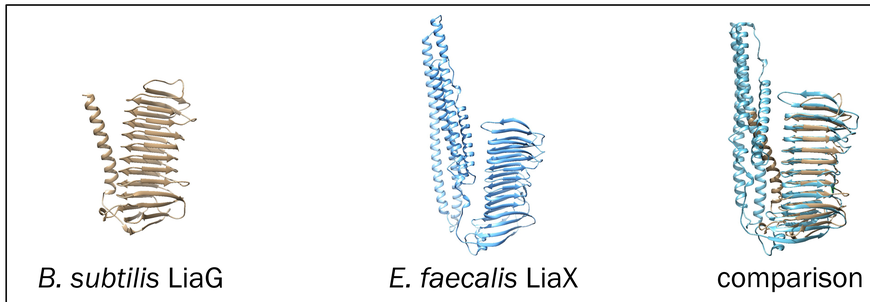

Figure 3. Protein structural predictions for Bsu LiaG (left) and Efs LiaX (middle). LiaG is predicted to have an N-terminal TM helix connected to DUF 4097. LiaX is predicted to have N-terminal alpha-helical domain connected to DUF 4097. Predictions were compared to produce an overlay showing their similarity

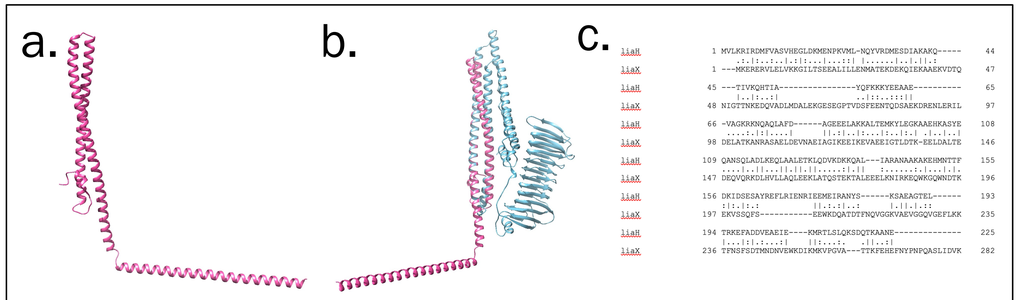

Figure 4. (a) Protein structural prediction for Bsu LiaH and (b) threaded onto Efs LiaX for comparison. LiaH is predicted to be mostly alpha-helical, like the N-terminus of LiaX. The sequence comparison for LiaH and the N-terminal 282 amino acids of LiaX is shown in (c).

Results/Implications

Efs and *Bsu* LiaF are arranged similarly with 4 transmembrane helices (TMH) in the N-terminal domain (NTD) and a β-sheet (βS) C-terminal domain (CTD). We identified LiaX-like gene products upstream from *Bsu* LiaFSR. Analysis of the predicted structures of LiaIHG showed that LiaI is a peptide with 4 TMH and structurally like the N-terminus of *Bsu* LiaF. Also, LiaH contains 3 putative α-helices akin to the N-terminus of LiaX. LiaG has 1 TMH connected to a large βS domain-like the C-terminus of LiaX. Each protein shared structural and sequence similarity that was not identified through BLAST searches.

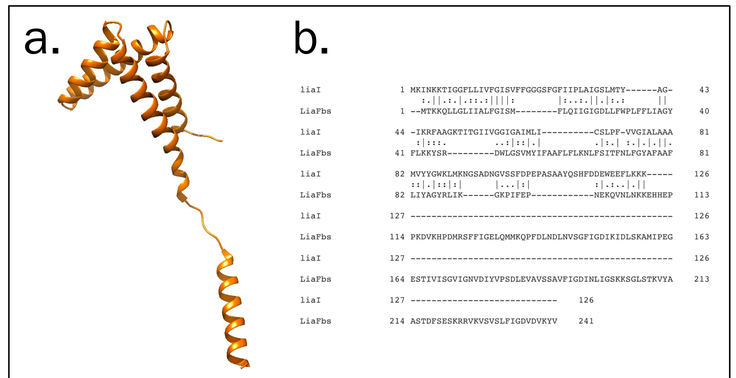

Figure 5. (a) Protein structural prediction for Bsu LiaI. Compare the structure, predicted to be 4 TM helices, to the N-terminal helices of Bsu LiaF in Fig. 1a. (b) Sequence alignment between Bsu LiaI and the N-terminus of Bsu LiaF

Future Actions

We identified *Bsu* LiaIHG as structural homologs to *Efs* LiaX, which was not previously known due to lack of sequence identity and empirical structural data. The findings further supports our model for LiaX and LiaF as core pieces in DAP-R development.

Acknowledgments

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