Rationally Designing the Most Potent Agonists and Antagonists for the *Enterococcus faecalis* fsr Circuit: Improving Attenuation of Quorum Sensing-Dependent Pathogenicity

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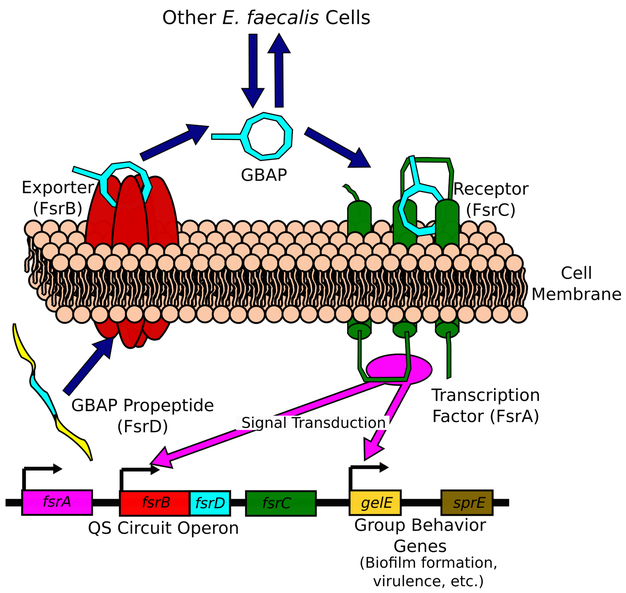
Introduction

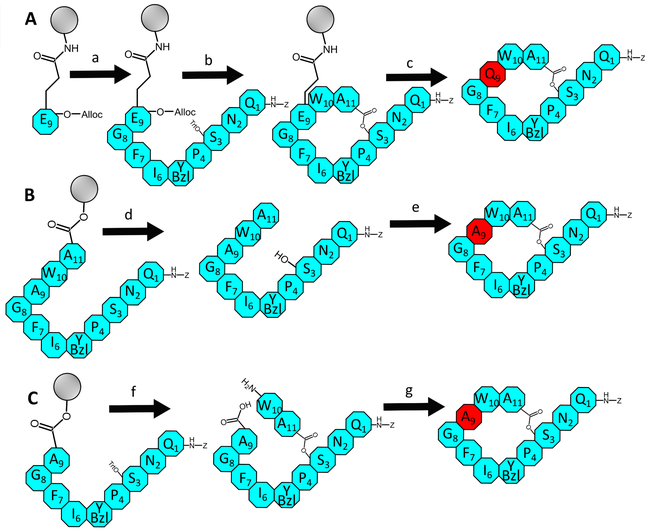
Resistance to antibiotics by bacteria remains a critical problem, particularly in cases of multi-drug resistance. *Enterococcus faecalis* is a common opportunist commensal gut bacterium, and is the leading cause of clinical enterococci infections. *E. faecalis* has exhibited both intrinsic and acquired antibiotic resistances and has been demonstrated to be capable of sharing antibiotic resistance with other bacterial species, such as Methicillin-resistant *Staphylococcus Aureus* (MRSA), increasing the prevalence of multi-drug resistant bacteria. Attenuating *E. faecalis* infections can thus not only decrease the costs associated with the initial infection, but can also help to prevent the spread of antibiotic resistance. An alternative approach to conventional antibiotics is to attenuate the pathogenicity of an organism, aiding the patient's immune system in clearing the infection while reducing the selective pressure for development of resistance to the treatment.

E. faecalis pathogenicity has been shown to be enhanced by activation of its fsr quorum sensing (QS) circuit. The auto-inducing peptide (AIP) used by *E. faecalis* to activate this circuit is termed gelatinase biosynthesis activating pheromone (GBAP). Structure-activity relationship studies between GBAP and the fsr receptor conducted by us and others have established a basic understanding of components crucial for agonist and antagonist activity. In the presented study, we have rationally designed the most potent agonistic and antagonistic peptide analogs known to date while further increasing our understanding of the structural components necessary for interaction between GBAP and the fsr receptor. Our results have also suggested avenues for further improvement.



E. faecalis Fsr QS Circuit:

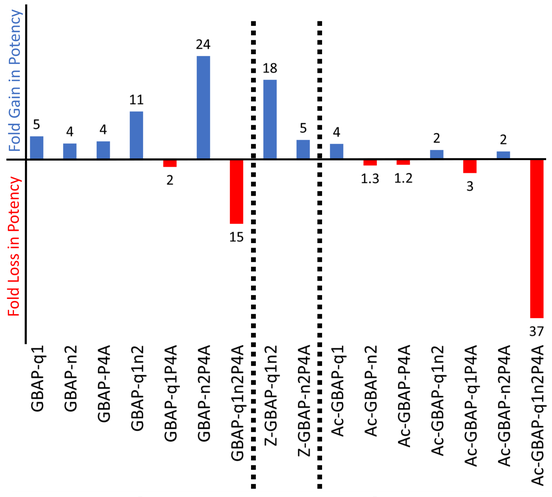
When GBAP binds to the membrane-bound histidine-kinase receptor FsrC, the transcription factor FsrA is phosphorylated, resulting in upregulation of: the exporter, FsrB, the GBAP propeptide FsrD, and FsrC (autoinduction). In addition, the group behavior genes gelE and sprE are upregulated, resulting in the production of virulence factors such as gelatinase and biofilm formation. The linear GBAP propeptide is processed into the mature macrocycle and exported by FsrB.



Synthetic Approaches:

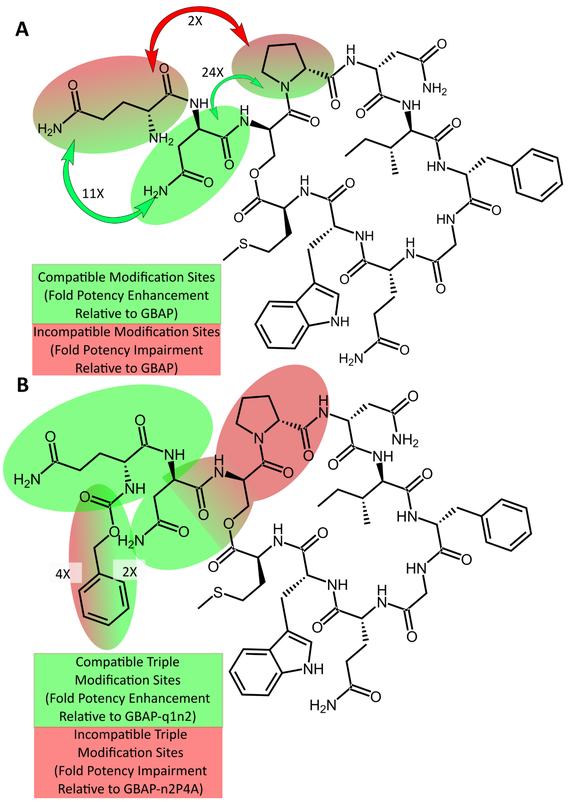
Most couplings used HATU-facilitated Fmoc-based SPPS. A) Entirely on-resin method: after attachment of protected glutamic acid *via* its side chain to rink amide MBHA resin, a) sequence is extended to the tail, b) selective deprotection of Ser3 side chain, on-resin ester formation, extension of remaining peptide sequence, and on-resin cyclization, c) final cleavage from the resin yields the desired peptide. B) In-solution ester cyclization: using Ala-loaded Wang resin, extend the sequence to tail, d) cleave the peptide from the resin, e) cyclize in solution *via* ester formation for final peptide. C) In-solution amide cyclization: using Ala-loaded Wang resin, extend the sequence to tail, f) selective deprotection of Ser3, on-resin ester formation, and extension of remaining peptide sequence, g) cleavage from the resin and subsequent cyclization in solution *via* amide formation.

Results



Potency Change Compared to GBAP:

Comparison of potencies of rationally designed agonist analogs. The fold change in potency of each analog is as compared with GBAP is shown. Loss of potency is shown in red, while gain in potency is shown in blue.



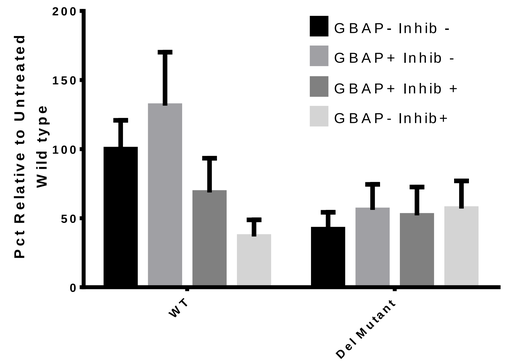
Effect of Addition of Z-group Demonstrates Modification Compatibility Differences:

Red shading indicates loss of potency while Green indicates the analog resulted in improved potency. (A) relative to GBAP or (B) relative to the most potent analog prior to capping the *N*-terminus with the Z-group.

GBAP-Based Antagonists

Sequence	IC_{50} [95% CI] (nM)
Inhibitor SAR Studies	
Z-QN(SP-**YBzl**-IFG**A**W**A**)	> 10,000‡, >10,000*
Ac-QN(SP-**YBzl**-IFG**A**W**A**)	> 10,000‡
Ac-(SP-**YBzl**-IFG**A**W**A**)	> 10,000‡
QN(SP-**YBzl**-IFGQW**A**)	139 [61.8 – 313] ‡
Z-QN(SP-**YBzl**-IFGQW**A**)	227 [172 – 299] ‡, 38.7 [26.8 – 55.9]*
[PyGlu]-N(SP-**YBzl**-IFGQW**A**)	295 [232 – 375] ‡
Ac-QN(SP-**YBzl**-IFGQW**A**)	444 [25 – 918] ‡
Ac-(SP-**YBzl**-IFGQW**A**)	872 [520 – 1463] ‡
QN(SP-**YBzl**-IFGQWM)	438 [225 - 853] ‡
Designed Inhibitors	
Z-qn(SP-**YBzl**-IFGQW**A**)	388 [183 – 826] ‡
Z-Q**n**(S**A-YBzl**-IFGQW**A**)	> 10,000‡
qn(SP-**YBzl**-IFGQW**A**)	967 [729 - 1281] ‡
Q**n**(S**A-YBzl**-IFGQW**A**)	> 10,000‡
qn(SP-**YBzl**-IFGQWM)	> 10,000‡
Q**n**(S**A-YBzl**-IFGQWM)	> 1,000‡

GBAP Competitor Conc. (‡50 nM | *5 nM)



Most Potent Inhibitor Attenuates Biofilm Formation:

The wild type biofilm production is compared with a deletion mutant that cannot quorate. Cells were treated with QN(SP-**YBzl**-IFGQW**A**) at a concentration that is 5-fold its IC_{50}. 50 nM of exogenous GBAP was added as a competitor. All results are normalized relative to the untreated wild type biofilm production.

Conclusions

- Rational design proved effective for development of more potent agonists, although several modifications were found to be incompatible with each other.
- The modifications that improved agonist activity were unfortunately incompatible with the modifications required to produce an antagonist.
- This study has identified the most potent agonists and antagonists for the *E. faecalis* fsr QS circuit known to date.
- The peptides developed are potent enough to allow more in depth bioactivity studies to be conducted – such as being tested in *in vivo* model organisms.

References and Acknowledgements

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