



Discovery of a potential target for the development of therapeutic peptides for preventing bacterial-mediated colorectal cancer

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Introduction

Many streptococci communicate by producing a signaling peptide, termed competence-stimulating peptide (CSP), to activate genes involved in group behaviors such as biofilm formation or the production of virulence factors, when the signaling molecule reaches a certain threshold concentration corresponding to high population density (Figure 1).¹ This method of bacterial communication is called quorum sensing (QS) and it allows a specific group of bacteria to activate certain genes that are vital for their survival.² *Streptococcus gallolyticus* subsp. *gallolyticus* (Sgg), a member of the group D streptococci, has been shown to promote human colon cancer cell proliferation, establishing Sgg as a bacterial driver of colorectal cancer (CRC).³ Our lab has identified Sgg CSP signal and discovered that it regulates the production of bacteriocins which helps Sgg outcompete other bacteria. We are currently working on determining the molecular mechanism that drives this QS circuitry, specifically, the CSP:ComD interactions. This communication presents our initial structure-activity relationship (SAR) results.

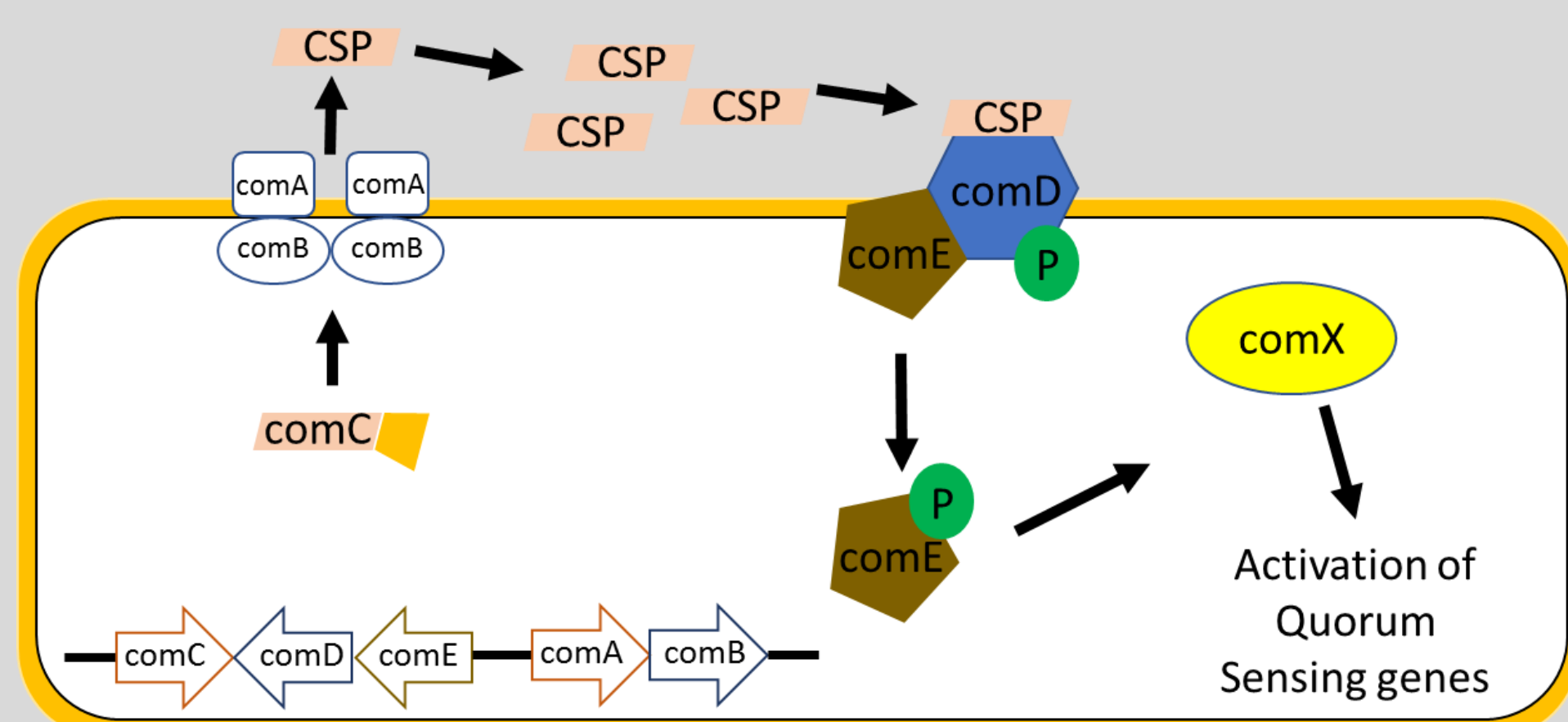


Figure 1. Diagram of Streptococci quorum sensing pathway

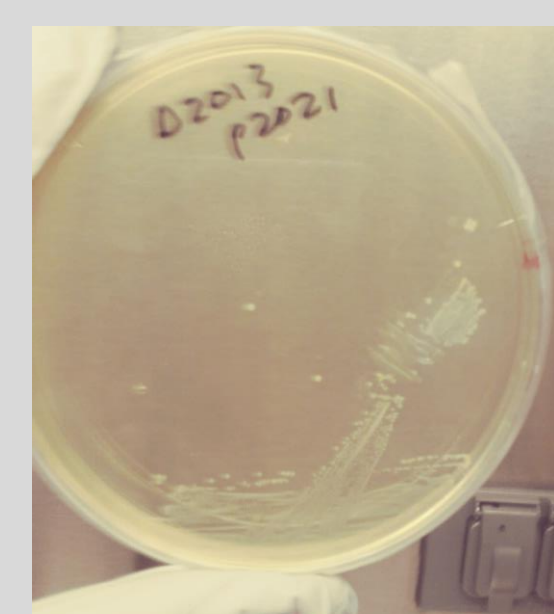
Approach & Methodology

1. *S. gallolyticus* subsp. *gallolyticus*_ATCC_43143
2. *S. gallolyticus* subsp. *gallolyticus*_DSM_16831
3. *S. gallolyticus* subsp. *gallolyticus*_UCN34
4. *S. gallolyticus* subsp. *gallolyticus*_TX20005
5. *S. gallolyticus* subsp. *gallolyticus*_NTS_31301958
6. *S. gallolyticus* subsp. *gallolyticus*_NTS_31106099
7. *S. gallolyticus* subsp. *gallolyticus*_NTS_31307555
8. *S. pneumoniae*_TIGR4
9. *S. pneumoniae*_D39
10. *S. intermedius*_NCDO2227

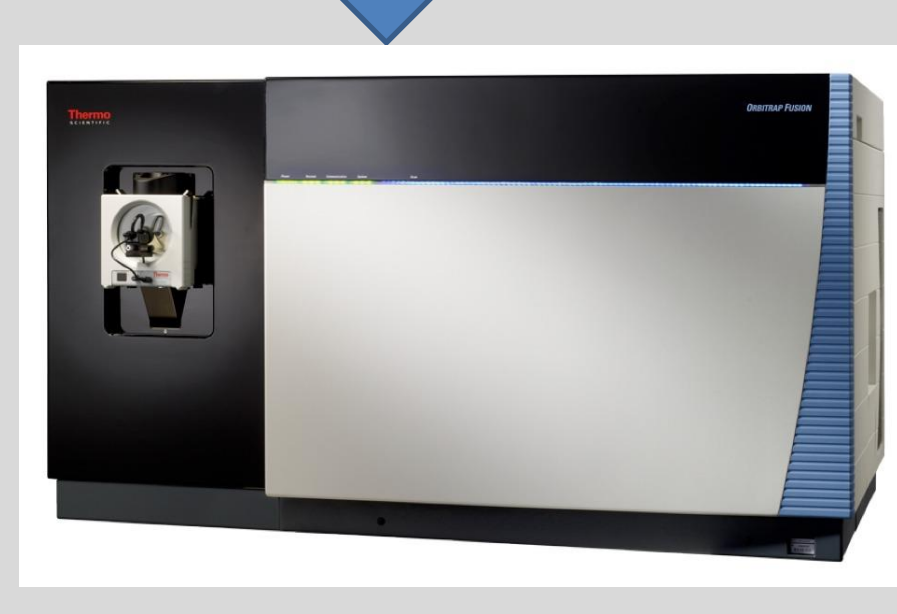
Predict amino acid sequence of CSP by multiple sequence alignment



Isolate natural CSP and synthesize predicted CSP using 9-Fluorenylmethoxycarbonyl (Fmoc) solid phase peptide synthesis



Conduct phenotypic assay to find the function of the signaling peptide



Tandem Mass Spectrometry (MS²) Peptide Mapping with Thermo Scientific™ Orbitrap Fusion™

Test analogs to determine the structure activity relationship

Results

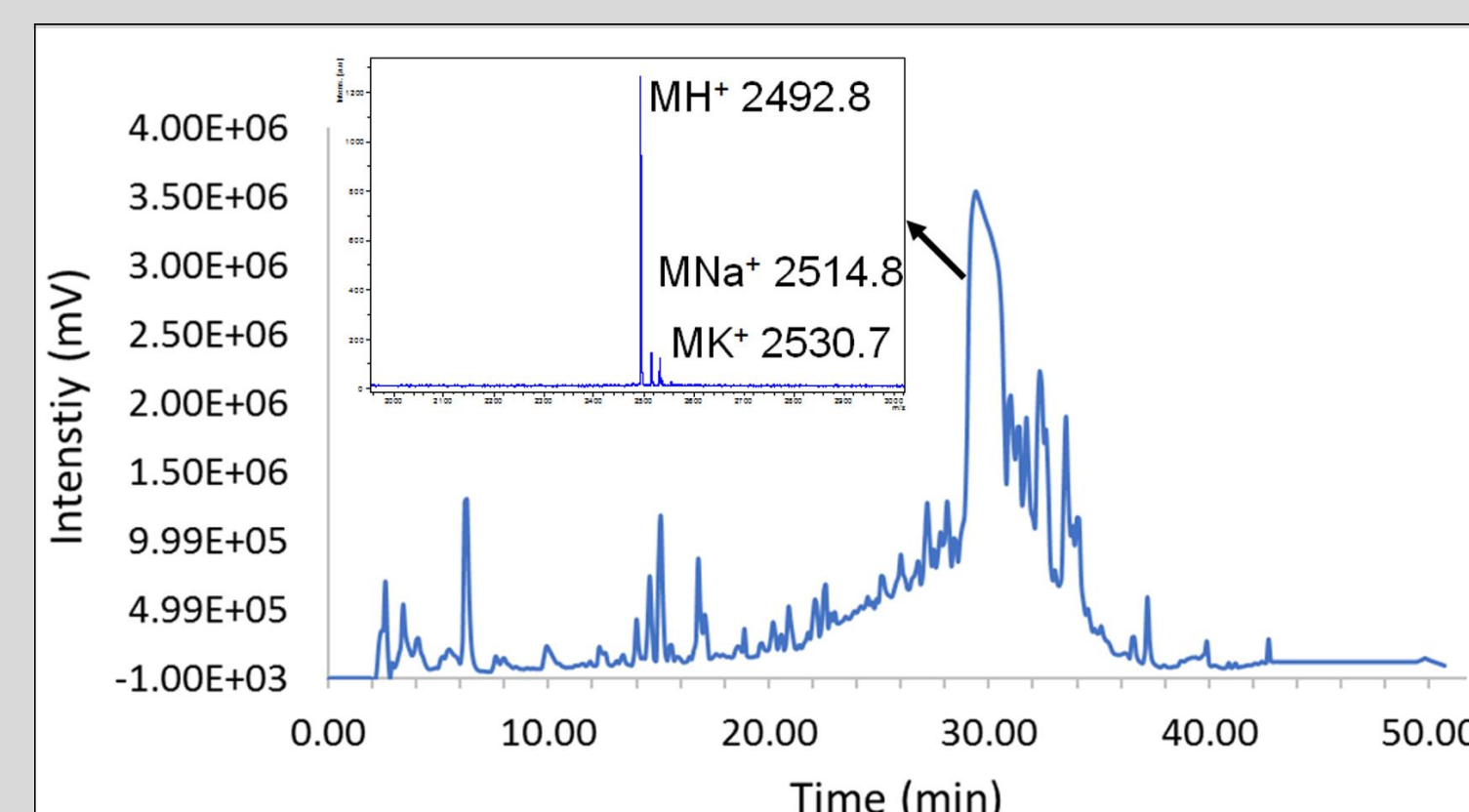


Figure 1. Isolation and identification of Sgg CSP. RP-HPLC chromatogram trace of crude protein extract from cell-free supernatants and MALDI-TOF MS of a fraction collected between 29.5 to 30.5 min (major peak).

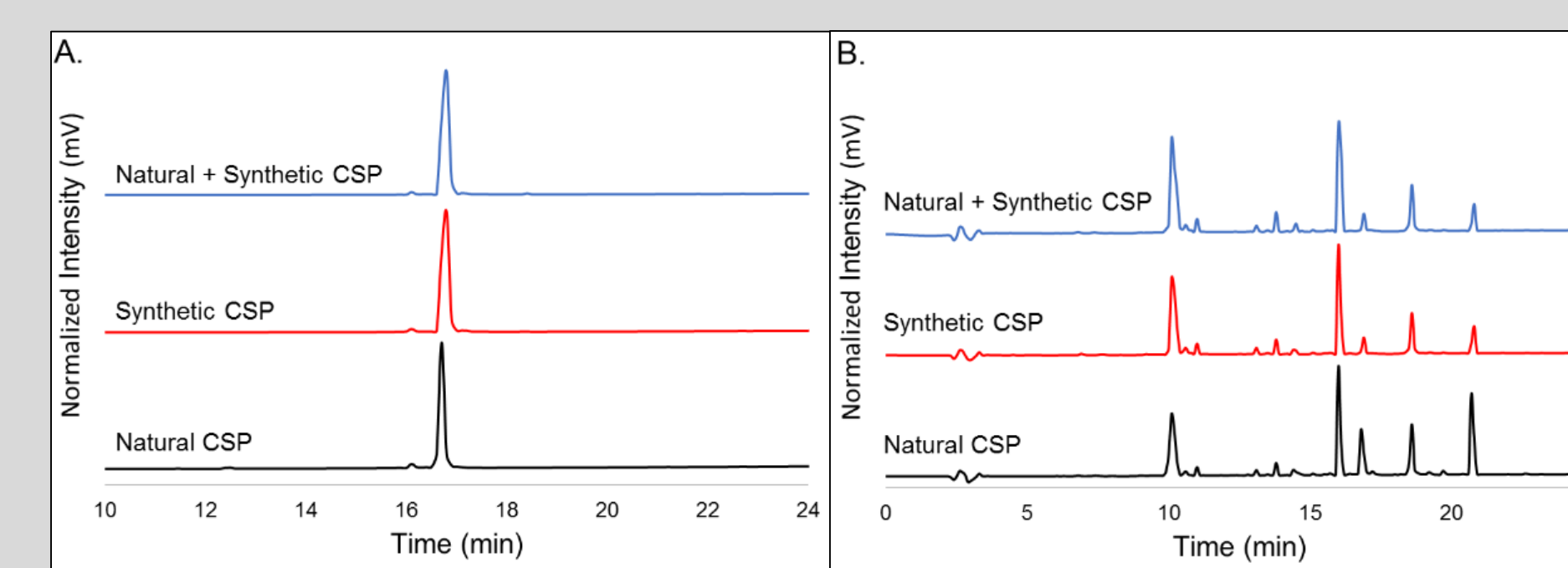


Figure 2. Comparison of synthetic and isolated CSPs. (A) Overlay with offsets of analytical RP-HPLC chromatograms of purified natural, synthetic, and natural and synthetic CSP. (B) Overlay with offset of analytical RP-HPLC chromatograms of chymotrypsin digestion of natural, synthetic, and natural and synthetic CSP. Tandem MS results confirmed our predicted sequence but was not confident about the first 2 residues.

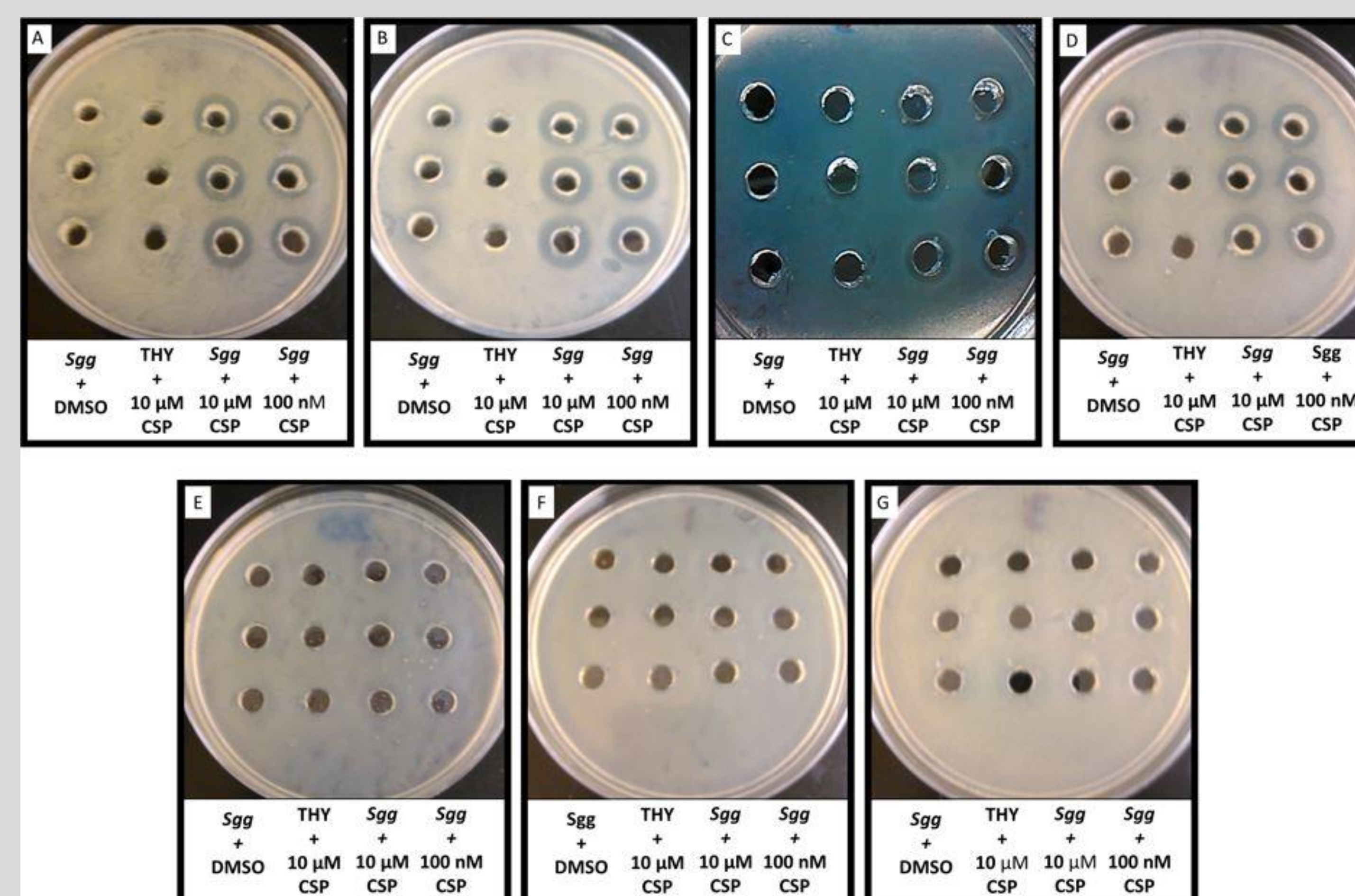


Figure 3. Images comparing results from Sgg interspecies inhibition assay, where Sgg is incubated with CSP (10 μM or 100 nM) or DMSO and the supernatants are tested against A) *S. anginosus* ATCC 33397 B) *S. constellatus* ATCC 27823 C) *S. vestibularius* F0396 D) *S. intermedius* F0413 E) *S. mutans* ATCC 25175 F) *S. agalactiae* MNZ938 G) Sgg TX20005. Note that part C looks different because the image was taken against a different background for better visualization.

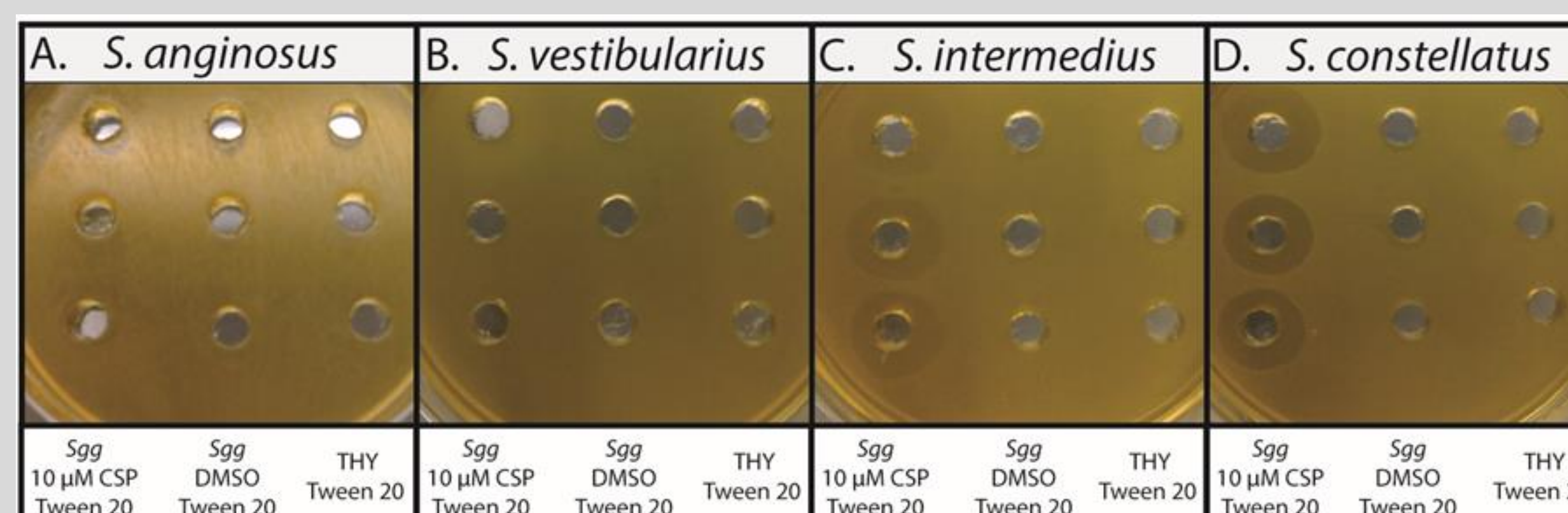


Figure 4. Images comparing results from Sgg interspecies inhibition assay testing sterile-filtered supernatants showing that the CSP regulates the production of a bacteriocin-like inhibitory substance.

Conclusions

- Sgg produces a 21-mer CSP, DFLIVGPFDWLKKNHKPTKHA, signal that regulates two mechanism of inhibition against other bacteria. One mechanism requires direct cell contact while the other mechanism is the production of a bacteriocin-like inhibitory substance.
- The alanine scanning reveals that there is no single residue that is critical for activation of comD (unpublished data not included).

Future Plans

- Perform reverse alanine scan to find a good receptor binding scaffold.
- Conduct phenotypic assays on any lead inhibitory analogs.

Acknowledgments

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References

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2. Bassler, B. L., How bacteria talk to each other: regulation of gene expression by quorum sensing. *Current opinion in microbiology* 1999, 2 (6), 582-587.
3. Kumar, R.; Herold, J. L.; Schady, D.; Davis, J.; Kopetz, S.; Martinez-Moczygemba, M.; Murray, B. E.; Han, F.; Li, Y.; Callaway, E., *Streptococcus gallolyticus* subsp. *gallolyticus* promotes colorectal tumor development. *PLoS pathogens* 2017, 13 (7), e1006440.
4. Harrington, A.; Tal-Gan, Y., Identification of *Streptococcus gallolyticus* subsp. *gallolyticus* (biotype I) competence stimulating peptide pheromone. *Journal of bacteriology* 2018, 200 (14), e00709-17.