

Analysis of Novel Streptococcal Probiotic Candidates' Antimicrobial and Anti-Inflammatory Properties

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Abstract

Background/Objective: The oral microbiome consists of intricate interactions between organisms that when out of balance can be a primary factor in the development of oral disease. An option for reducing the risk of oral disease is to identify probiotic strains that inhibit the growth of oral pathogens or in some way promote oral health. We hypothesize that bacterial strains isolated from children with a caries free history will be a source of probiotic candidates that inhibit the growth of cariogenic or periodontal pathogens or suppress chronic inflammation.

Methods: Antagonistic effects of candidate probiotic strains were assessed by measuring zones of inhibition when grown in close proximity to pathogenic (*S. mutans*, *S. sobrinus*, *C. albicans*) or health-related (*S. sanguinis*) oral microbes. Quantitative Polymerase Chain Reaction was used to quantify relative expression of pro- and anti-inflammatory cytokines in co-cultures of probiotic candidates and mouse macrophage RAW 264.7 cells.

Results: Two (HP3 and HP5) of ten mildly acidogenic probiotic candidates were found to possess strong antagonistic activity towards *S. mutans*. Four (IOWA1-4) other probiotic candidates, previously determined to inhibit *S. mutans*, significantly elevated expression of the anti-inflammatory cytokine IL-10 in a mouse macrophage co-culture.

Conclusion: The data from the candidates' antagonism of *S. mutans* and influence on cytokine expression supports the hypothesis, but the effectiveness and limitations of each candidate strain vary suggesting that the most effective approach to promote oral health via probiotics will be a combination of strains with diverse properties.

Background & Objectives

- The two leading causes of poor oral health are periodontal disease and caries².
- Probiotic strains may inhibit the growth of oral pathogens or in some way promote oral health.
- Aim 1: Testing for the inhibition of oral pathogen growth in the presence of candidate probiotic strains obtained from children with a caries-free dental health history.
- Aim 2: Testing for anti-inflammatory properties of candidate strains.
- Hypothesis: Bacterial strains isolated from children with a caries free history will be a source of probiotic candidates that inhibit the growth of cariogenic or periodontal pathogens or suppress chronic inflammation.

Materials and Methods

Aim 1

Culture

- Previously isolated bacterial strains
- BHI plates

Suspension

- PBS buffer

Adjust suspension concentration

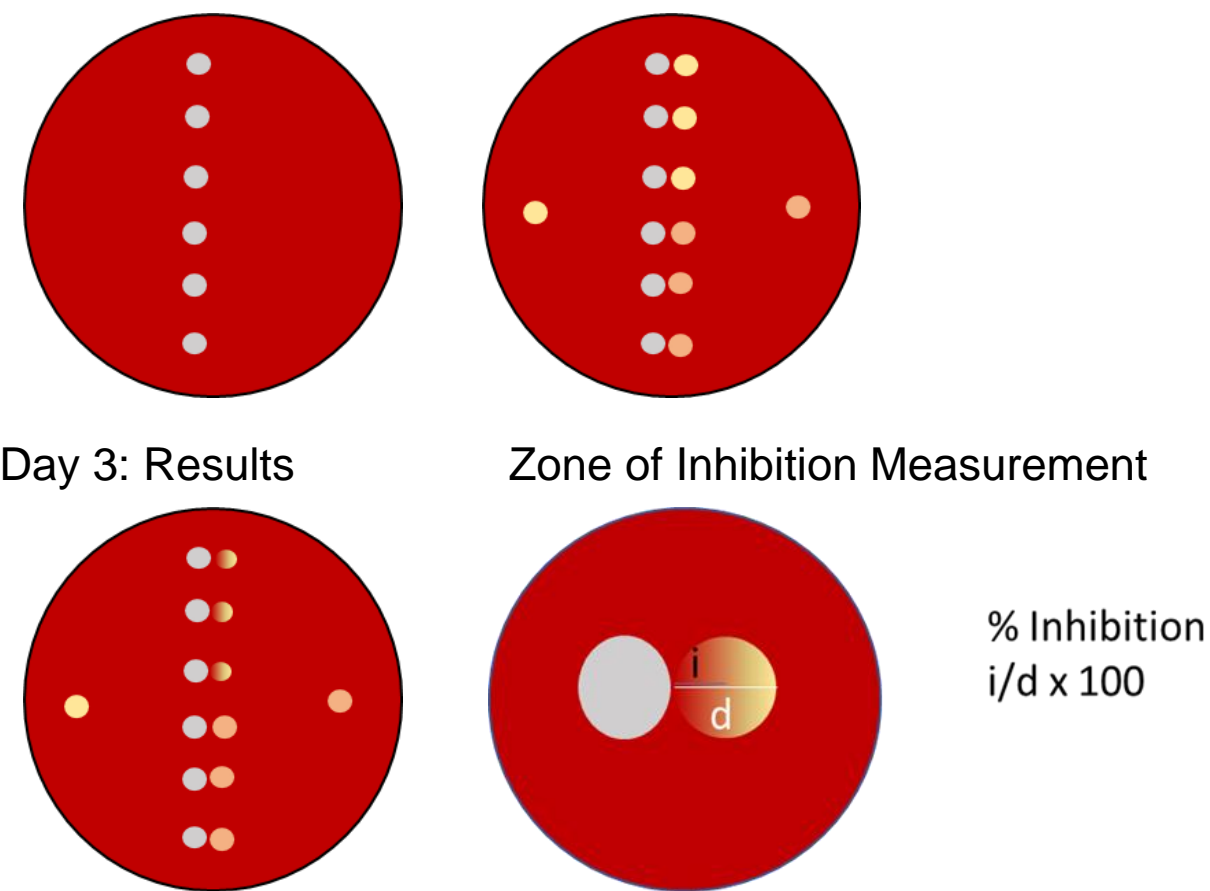
- Spectrophotometer to measure turbidity @ 600nm
- OD = 0.100 A +/- 0.005

Inoculate

- 7µl inocula

Plating Order

Day 1: Initial Inoculation Day 2: Secondary Inoculation



Aim 2

RAW 264.7 cells of mouse macrophage cell line

- Co-culture with IOWA1-4 candidate strains on DMEM
- Removed from incubation at 6 and 24 hours

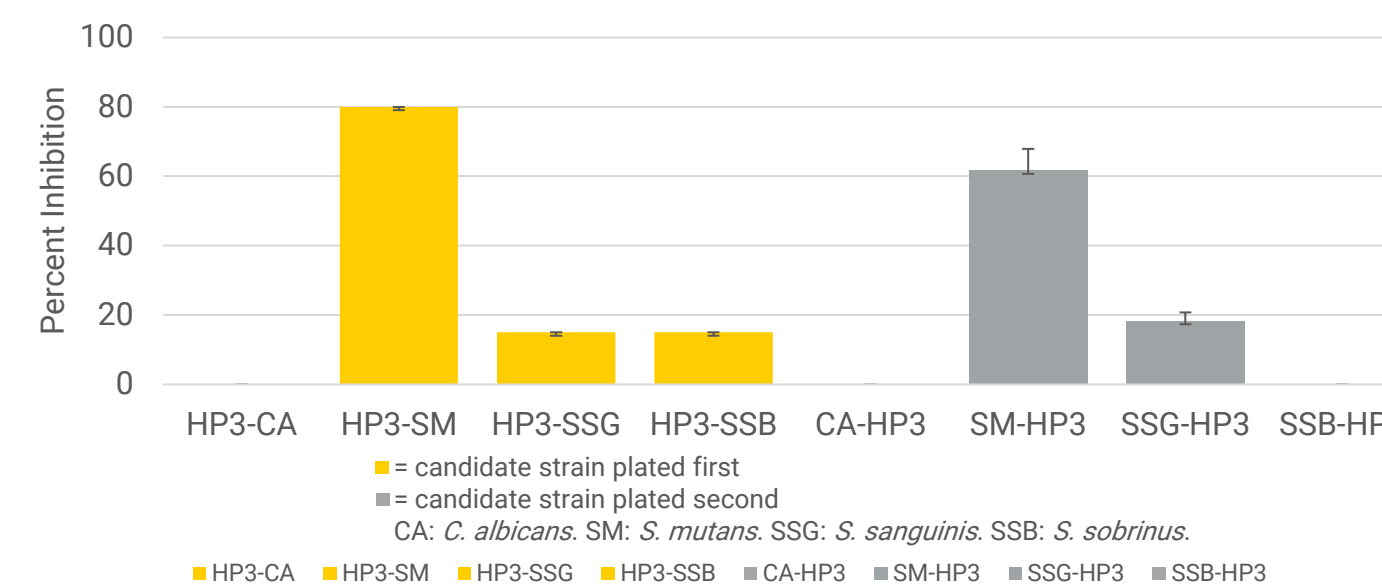
QPCR

- Direct-zol™ RNA MicroPrep kit from Zymo Research used to isolate RNA
- Primers for common cytokines associated with inflammation (IL-1B,6,8,10,17)

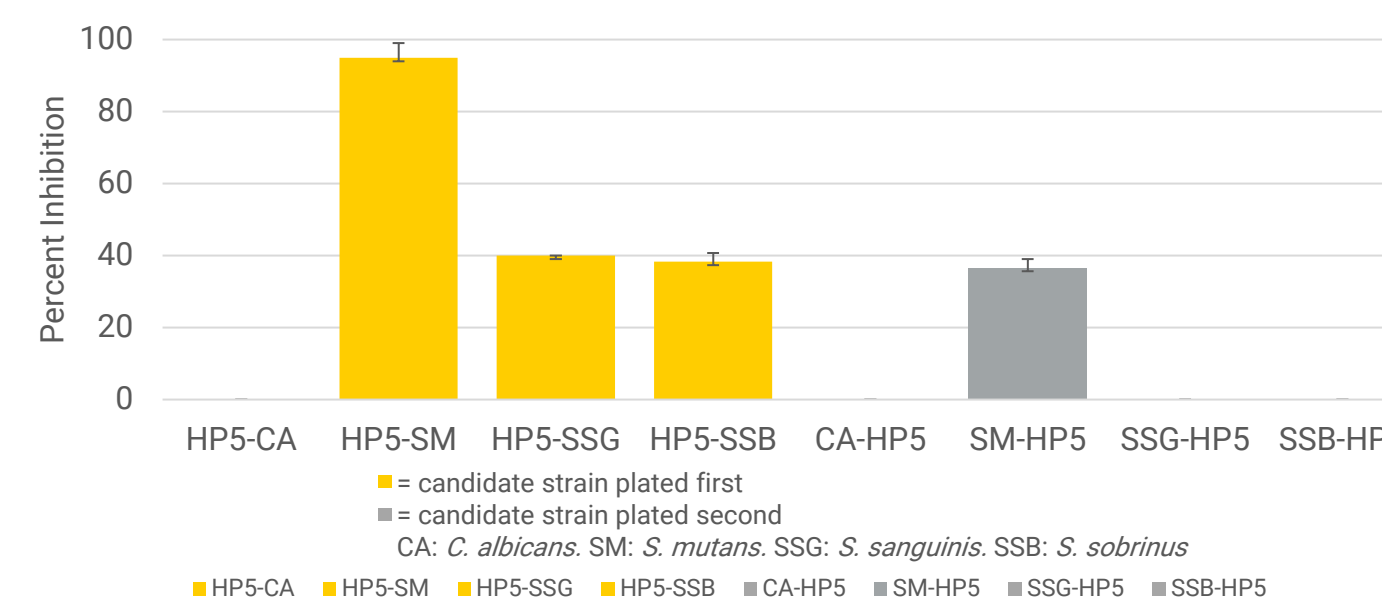
Results

Aim 1

HP3 Candidate Strain

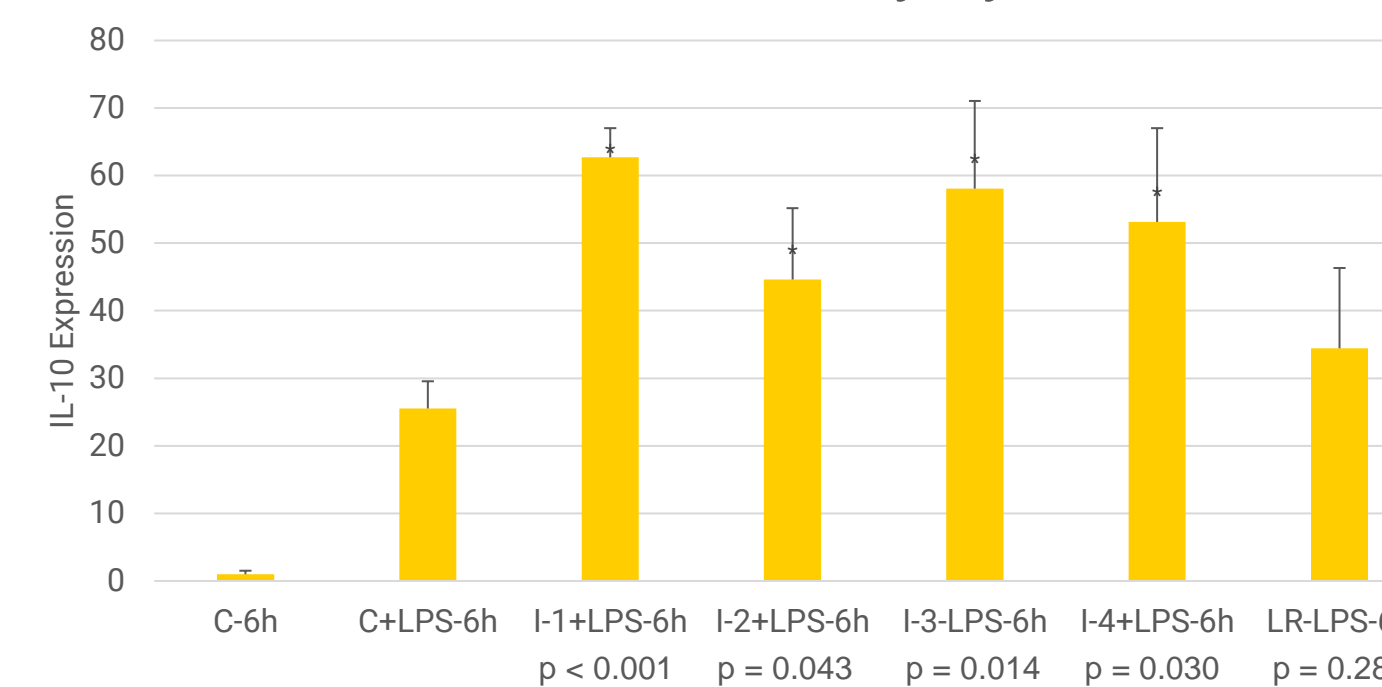


HP5 Candidate Strain



Aim 2

IL-10 Anti-Inflammatory Cytokine*



C: only macrophage cells to normalize expression values. C+LPS: LPS stimulates inflammatory response and used this as comparison control. I-X+LPS: candidate strain and LPS. LR+LPS: Lactobacillus reuteri + LPS used as current oral probiotic comparison.
* IL-10 is an anti-inflammatory cytokine; elevation of IL-10 expression leads to decreased inflammation. It also plays an important role in inhibiting bone resorption⁷.

Conclusions

- HP3 and HP5 antagonism of *S. mutans* and the influence of IOWA1-4 on IL-10 expression both support the hypothesis.
- Effectiveness and limitations of each candidate strain vary.
 - We suggest the most effective approach to promote oral health via probiotics will be a combination of strains with diverse properties.

References

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