Poster # 2362

Inulin, Xylitol and Zinc is Prebiotic and Promotes Fresh Breath

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Objective

We developed a prebiotic dentifrice formula containing inulin, xylitol and zinc in order to promote breath health through microbiome shifts. We studied the ability of these ingredients alone and in combination to shift the quantity and composition of *in vitro* oral microbial communities. We then explored the impact of brushing with this formula on breath quality and health, as well as salivary microbiome.

Conclusions

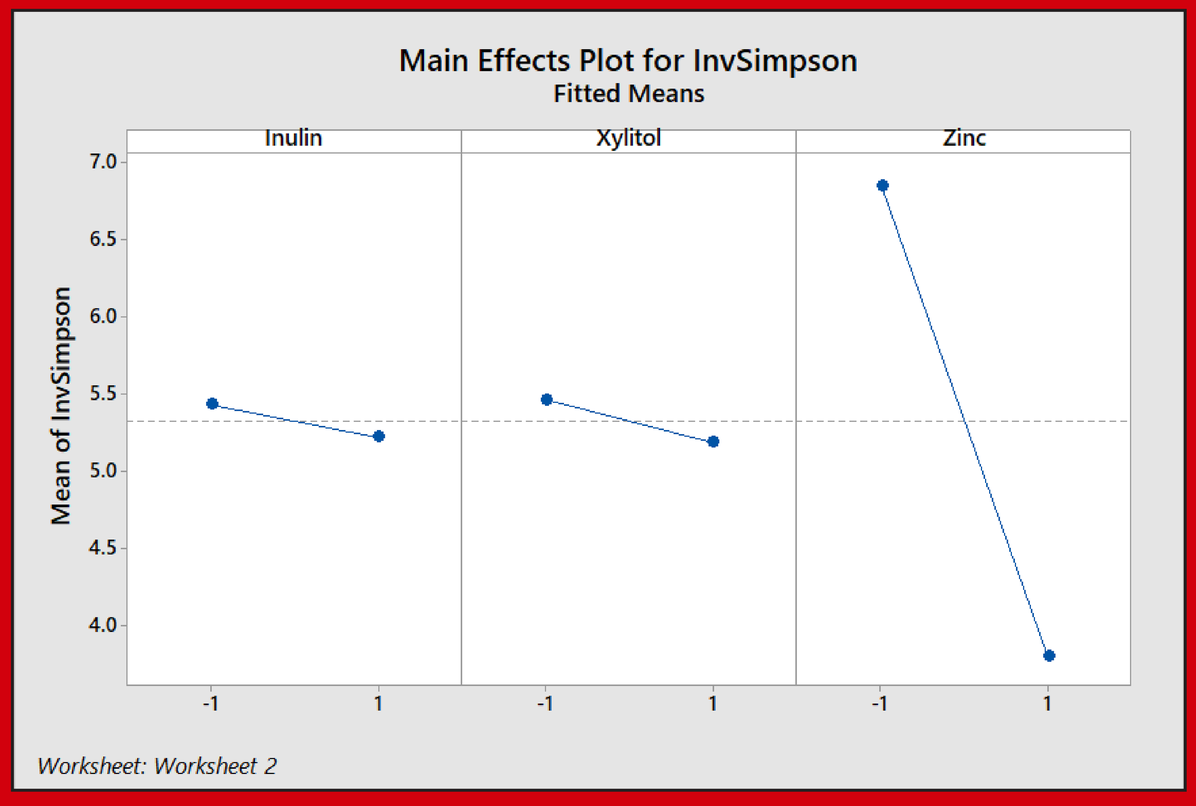
Based on the data presented here, we propose the following role for the three ingredients in our inulin, xylitol and zinc containing toothpaste:

- Zinc exerts a broad-spectrum effect reducing the metabolic activity of the oral microbiota making bacteria more susceptible to shifts.
- Xylitol inhibits acid-producing bacteria, minimizing potential effects from free monomer contamination in the inulin.
- Inulin promotes the growth of beneficial organisms while reducing the growth of organisms associated with damaging malodor compounds.

Results

Figure 1. (A) DOE analysis of the effects of the three ingredients alone or in combination on the quantity and diversity of *in vitro* oral biofilms. The largest effect on biofilm diversity was driven by the zinc. Xylitol and inulin have smaller, but significant impacts and increase the diversity of oral biofilms. None of the ingredients appear to interact. (B) Looking at selected genera, those associated with malodor are generally decreased in biofilms treated with the three ingredient combination. These results also demonstrate the differential effects of the three ingredients on select genera.

A



B

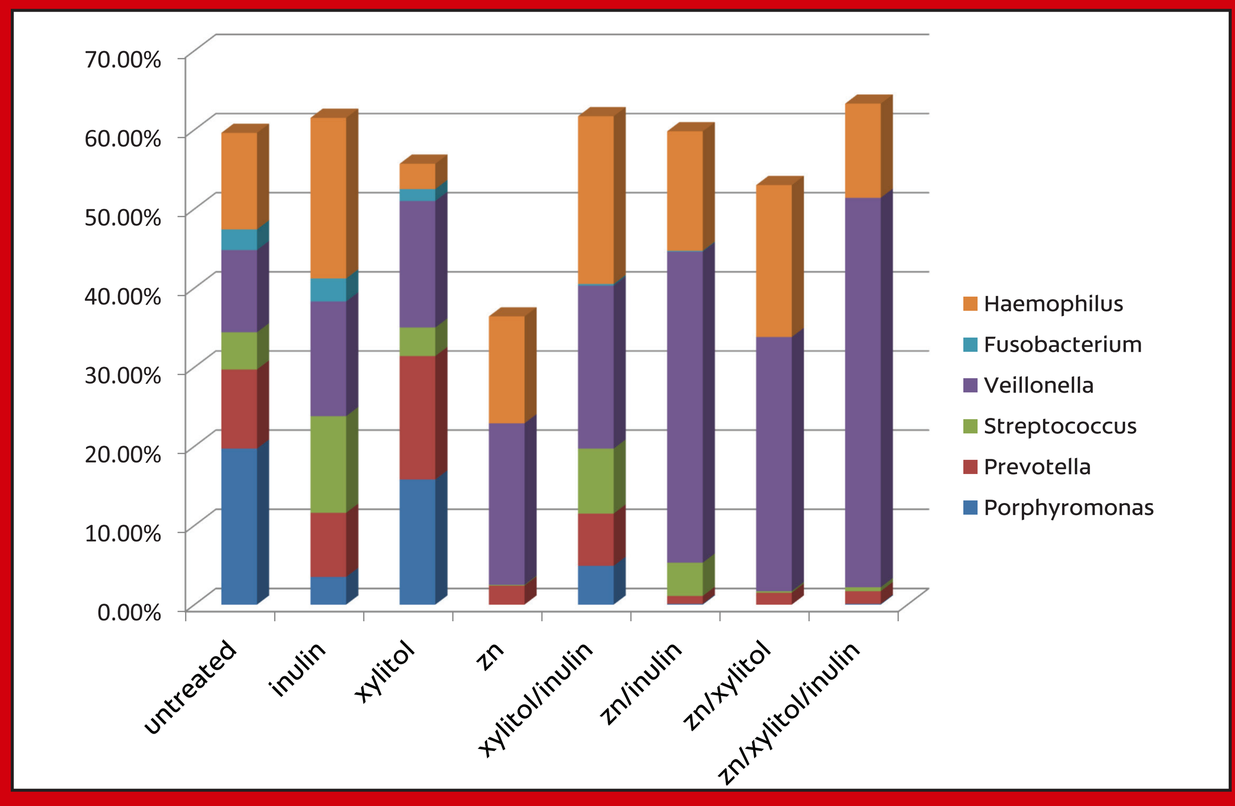
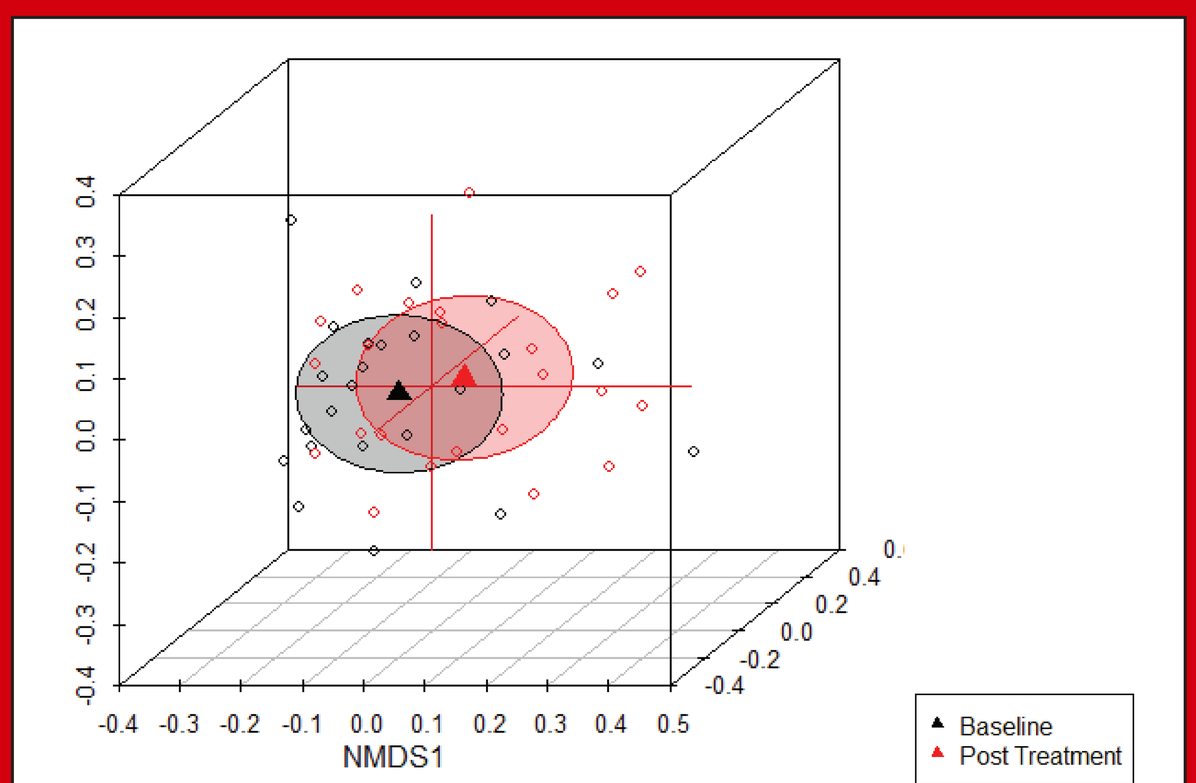


Figure 2. Clinical impact of inulin toothpaste on salivary microbiome. Panelists brushed for a total of 3 weeks with an inulin/xylitol/zinc containing dentifrice. (A) A clear shift in the salivary microbiome can be seen between baseline and post-use of the dentifrice. (B) Key genera involved in oral malodor decreased, while two genera associated with oral health increased following use.

A



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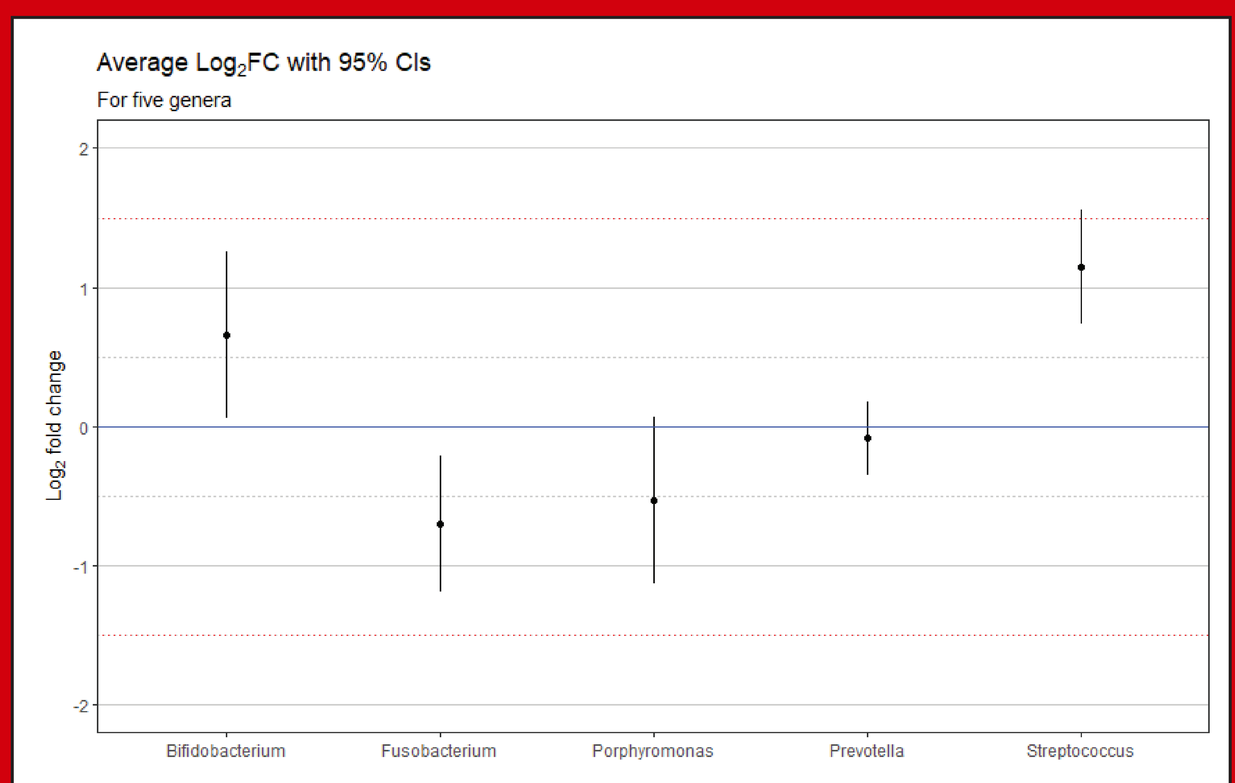

Methods and Materials

- *In vitro* biofilms
  - Saliva-derived biofilms grown for a total of 108 h on vertical HAP discs, SHI media incubated in 5% CO_2
  - Treated 2x/day with at least 6 h between treatments for a total of 9 treatments
  - Biofilms harvested, total biomass and ATP production measured
- 16s rRNA sequencing
  - Both *in vitro* biofilms and clinical saliva samples were tested
  - DNA was extracted using the DNeasy Power Biofilm kit.
  - DNA was amplified using the Roche FastStart High Fidelity PCR system to amplify the V3-V4 region.
  - Mothur pipeline was used to process the raw reads. Updated HOMD database was used for taxonomic classification.
- Design of Experiments
  - A 2^3 factorial design to look at ingredient interactions within the tested formulas.
  - All possible combinations of the three ingredients as either present or absent were formulated. Analysis performed in Minitab.
- Clinical study design
  - 24 subjects, 18-70 years of age with VSC level of > 50 ppb
  - Randomized, double-blind, clinical study to evaluate the reduction in VSC after use of inulin prebiotic versus a negative control dentifrice
  - Assessments: Breath analysis (Syft Voice 200) for 18 volatile organic compounds, including VSCs and saliva collection at weeks 1 and 3

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