The Development of an Assay to Screen Potential Prebiotics for Use in Oral Health Care Solutions

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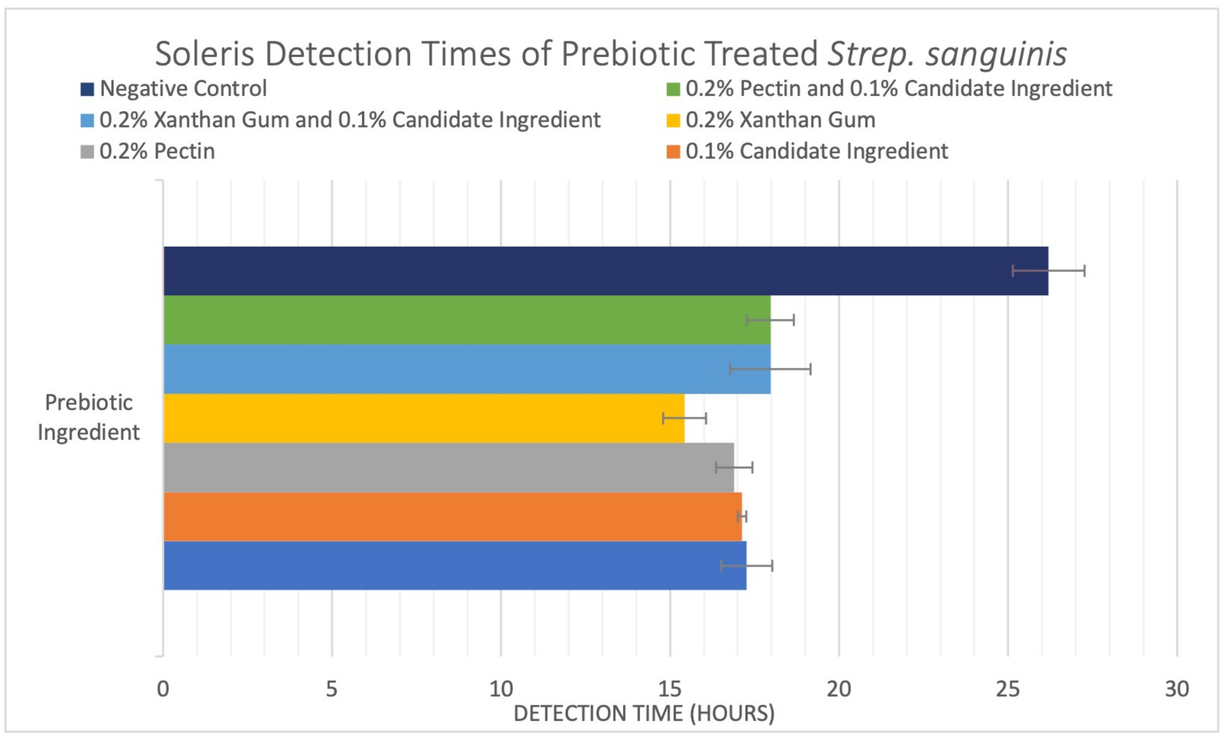
Gingivitis is an inflammatory response driven by the accumulation of plaque and the subsequent dysbiosis of the gingival microbiome. Treatments that could increase the resilience of an individual's microbiome against dysbiosis would be transformative to the oral health category and on the global burden of disease as periodontal diseases affect 750,847 million people yearly[1]. Using prebiotics could help shift the microbiome towards health. There is a lack of a simple, yet relevant screening method for prebiotics against oral bacteria. This is in part because of the complexity of the oral microbiome. This project aimed to define a robust protocol that could be used in-house within the limited microbiology resources available in house at GlaxoSmithKline Weybridge.

Background: A healthy gingival bacterial microbiome is difficult to define however, health associated bacteria are often Gram-positive, facultative anaerobes[2] and includes *Streptococcus sanguinis.* Dysbiosis towards a more proteolytic phenotype is associated with disease and Gram-negative obligate anaerobes[2] such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* [3] are often present. The relationship between organisms within the oral biofilm and with the host are complex and multifactorial, commensals play a role in maintaining health and can resist against un-favourable colonisers. Therefore, maintaining low levels of high quality 'healthy' plaque is paramount for oral health.

Prebiotics are non-digestible substrates that promote the growth of beneficial bacteria to give a health benefit[2]. Research on prebiotics for oral health is limited and many of those discovered are patent protected. The most well defined oral health prebiotics include inulin [4] , N-acetyl-D-mannosamine, β-methyl-D-galactoside [5], mannan-oligosaccharides (MOS)[7] and gums including xanthan gum (XG) [6]. Confidential data from William Wade's group at Kings College London was also used to define a candidate prebiotic ingredient.

Methods: Aerobic bacterial growth can be colorimetrically quantified using a Soleris® machine. A detectible threshold of colour change in the indicator at the base of the vials signifies the start of bacterial exponential growth. The prebiotic potential of selected 'ingredients' was quantified by aerobically incubating 0.5mL of *Strep. sanguinis* (OD_{550} 0.136 (-4 dilution)) with 0.5mL of a prebiotic at 37°C for 72 hours in the Soleris®. The experiments were repeated in triplicate and detection times were analysed using a student's t-test, to compare each treatment against the negative control (peptone water) and positive controls (inulin).



Graph 1: The detection times of prebiotic treated Strep. sanguinis compared to a negative control and 0.1% Inulin as the positive control.

Results: Preliminary Soleris® results showed all treatments significantly reduced the detection time when compared to the negative control. This could indicate that the ingredients were promoting bacterial growth and therefore could have prebiotic potential. The most significant decrease in detection time was seen with XG treatment however, when paired with the candidate compound the effect was reduced, suggesting an interaction between the two ingredients.

Conclusions and Discussion: Although the results are promising, to be able to validate that these ingredients are prebiotics their effect, if any, on periopathogens needs to be researched.

Developing a simple assay for such a complex system is difficult. The complexity arises from the microbial makeup of the oral microbiota; the mix of anaerobic and aerobic bacteria makes finding a universal assay to fit both these conditions challenging. The biggest limitation of the study is that the assay can only test the prebiotic potential of an ingredient against a single bacterial species. The interactions within the oral microbiome are essential and are likely to have a significant effect on the results and it is impossible to define a prebiotic from studies on single bacterium or even small communities [5], [8]. It is therefore not only essential to carry out such experiments but they will also give a better understanding of how these compounds would work *in-vivo.*

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