Balancing the oral microbiome based on the hydrogen peroxide-producing bacteria

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Objectives: Prebiotic oral care products with ingredients expected to encourage the function of beneficial bacteria and inhibit the growth of harmful bacteria are continuously being released. However, it is still unclear which bacteria are beneficial. In order to develop prebiotic oral care products, we tried to clarify the beneficial bacteria among the commensal bacteria and find some ingredients helpful for balancing the microbiome.

Methods: Oral probiotics such as *Lactobacillus reuteri* have known to produce hydrogen peroxide (H₂O₂), which can attack anaerobic pathogenic bacteria. Therefore, we defined and selected beneficial bacteria depending on their properties of H₂O₂ production by cultivating the *Streptococcus* species on the Prussian blue agar plate. Through understanding the feature of beneficial good bacteria, we developed a prebiotic oral care product and confirmed it was useful for balancing the oral microbiome.

Results: *S. gordonii*, *S. oralis* and *S. thermophilus* produced H₂O₂, otherwise *S. sanguinis*, *S. salivarius*, *S. mutans* did not. In addition, we found that H₂O₂ producing bacteria were present in the human saliva, but there was an individual difference indicating that oral environment could regulate the microflora. *S. gordonii* secreted H₂O₂ when it is superior to the competition with *S. mutans*. It was regulated by oral environments such as pH of saliva, energy sources, antibacterials and anti-gingivitis active ingredients.

Conclusions: Based on our results, we developed a toothpaste containing alternative energy sources; stevia and xylitol, a mild anionic surfactant; sodium methyl cocoyl taurate (SMCT) and anti-gingivitis active ingredients; *Centella asiatica* extract, *Zea mays* oil unsaponifiables, *Magnolia officinalis* bark extract. It was helpful for *S. gordonii* to prevail and produce H₂O₂ *in vitro*. Finally, we found that 2-week application of prebiotic toothpaste reduced the ratio of anaerobic periodontal pathogens; 81%, 42% and 52% reduction for *P. gingivalis*, *T. forsythia* and *T. denticola*, respectively.

Keywords: Hydrogen peroxide, *S. gordonii*, Stevia, Xylitol, SMCT, *Centella asiatica* extract, *Zea mays* oil unsaponifiables, *Magnolia officinalis* bark extract