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研究題目：Antimicrobial activity of green tea-derived epigallocatechin gallate on caries-associated bacteria

目的：

Diet is a key factor influencing oral health, thus more and more studies focus on the functions of food or additives in oral health. Green tea (a *Camellia sinensis* dry leaf infusion) is one of the most popular beverages in the world, and it is known to be a rich source of polyphenols, which have been reported to exhibit a wide range of biological properties, such as antioxidant, antimicrobial, anti-inflammatory, and cancer-preventive effects. Clinical trials have demonstrated that drinking tea (without added sugar) is associated with lower caries levels in humans. In addition, mouthwash with green tea (1.6 g green tea in 40 ml distilled water, 3 times per day for 1 week) significantly reduced the levels of the virulent cariogenic pathogens *Streptococcus mutans* and lactobacilli in saliva.

Four polyphenols are the major polyphenols in green tea, (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epigallocatechin-3-gallate (EGCG). EGCG is the most common polyphenol in green tea, accounting for > 50% of all green tea components, followed by EGC and ECG. EGCG also exhibits the strongest antimicrobial activity of all catechins and has been considered as the main anticaries component in green tea. It should be noted that mouth rinsing with green tea extract or EGCG solution for a short time is still effective in the reduction of cariogenic microbiota and the prevention of dentin erosion *in vivo*. However, these antimicrobial properties have not been characterized in detail, especially the effects of EGCG on sugar-induced acid production and the adhesion/aggregation of non-mutans

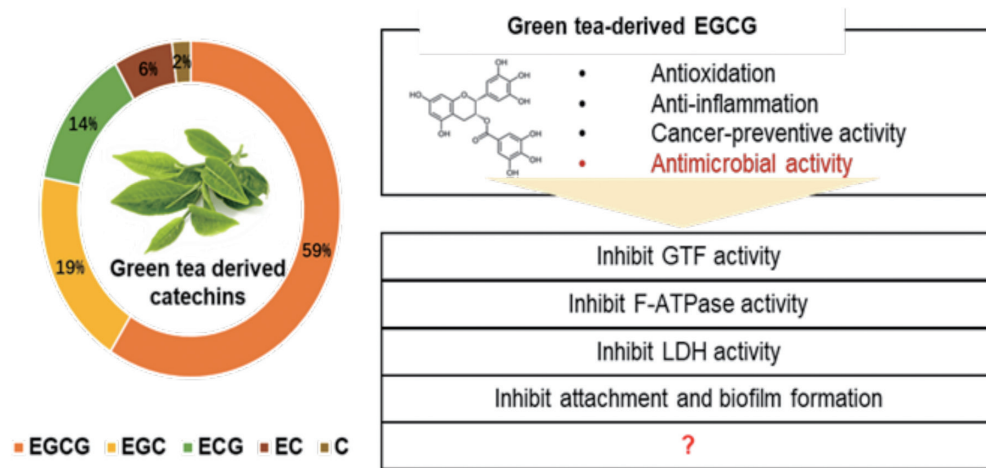


Figure 1. Research theme scheme

streptococci as well as mutans streptococci. Therefore, the present study aimed to characterize the effects of EGCG against monocultured planktonic mutans streptococci and non-mutans streptococci by examining its bactericidal activity ; its inhibitory activity against bacterial acidogenicity, acidic end-product production, and sugar uptake ; and its effects on bacterial aggregation, as well as the influence of saliva on these effects.

### 対象および方法 :

Representative caries-associated bacterial species, including *S. mutans* NCTC 10449 as a mutans streptococcus, and *S. sanguinis* JCM 5708, *S. gordonii* JCM 12995, and *S. salivarius* JCM 5707 as non-mutans streptococci, were used in this study. The antimicrobial properties of EGCG were evaluated by examining its bactericidal activity. The effects of EGCG on bacterial growth, acid production, acidic end-product formation, sugar uptake (phosphoenolpyruvate-dependent phosphotransferase system, PEP-PTS activity), and aggregation of streptococci were also evaluated. To explore the influence of saliva, I further examined the antimicrobial properties of EGCG within stimulated saliva by generating experiments mentioned above (Fig. 2).

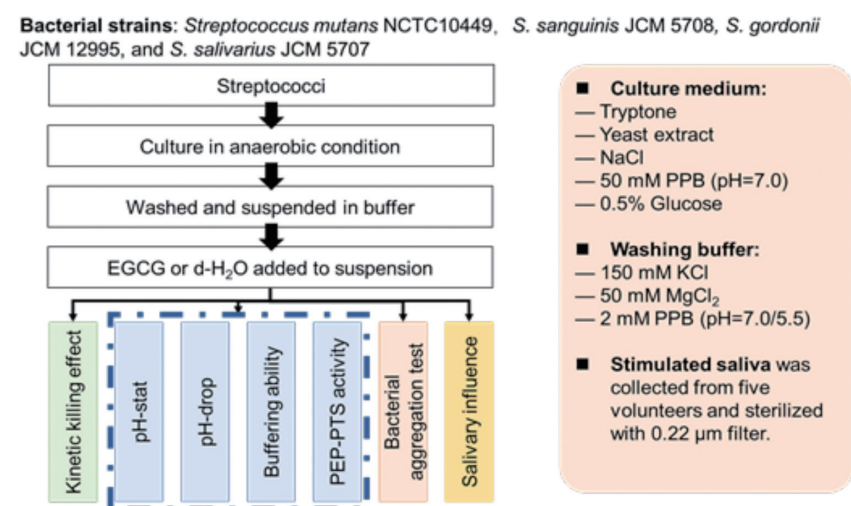


Figure 2 Research scheme

### 結果および考察 :

No bactericidal effects were detected after co-incubation of *S. mutans* with EGCG (1 mg/ml) for 4 hours while it decreased the growth and acid production of *S. mutans* (Fig. 3) by inhibiting the activity of the PEP-PTS. 2-hour incubation with EGCG (2 mg/ml) caused rapid aggregation of bacterial cells and reduced the optical density of *S. mutans* cell suspension by 86.7% at pH 7.0 and 90.7% at pH 5.5 (Fig. 4) . EGCG also reduced the acid production of non-mutans streptococci, including *S. sanguinis*, *S. gordonii*, and *S. salivarius*, and promoted the aggregation of these non-mutans streptococci. Furthermore, short-term EGCG treatment still effectively functioned in the presence of saliva.

In conclusion, firstly, EGCG inhibited the growth of *S. mutans*, a representative caries-associated microorganism with a high acidogenicity and aciduricity, at concentration of 1 mg/ml, and it had no effect on the viability of *S. mutans* except for 4 hour-treatment with 2 mg/ml EGCG. Secondly, EGCG possesses buffering capacity and inhibits bacterial acid production by reducing bacterial glucose uptake (PEP-PTS activity), both of which might coordinately

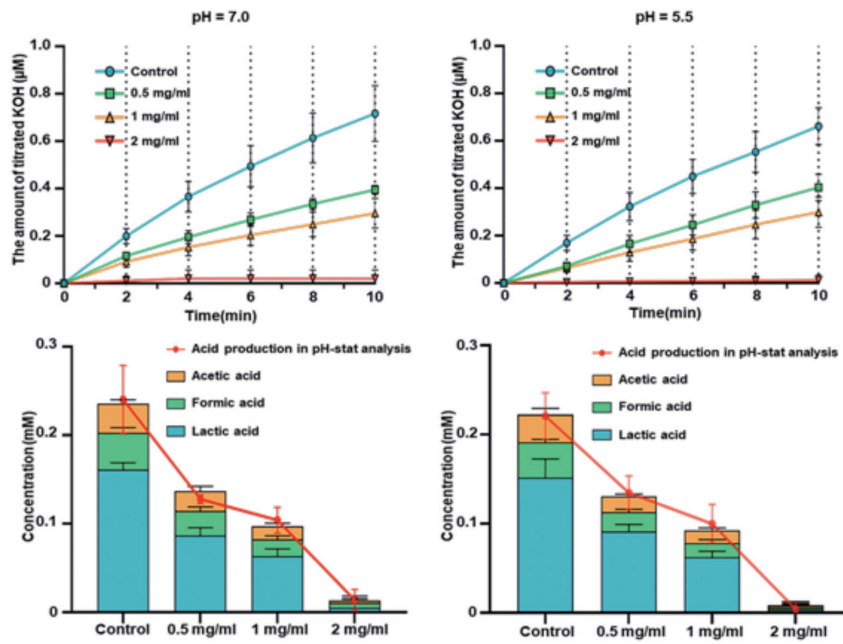


Figure 3. Effects of EGCG on the acid production of *S. mutans*

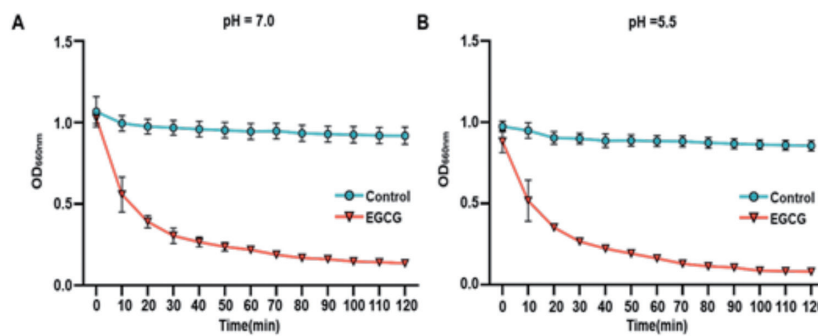


Figure 4. Effects of EGCG on the bacterial aggregation

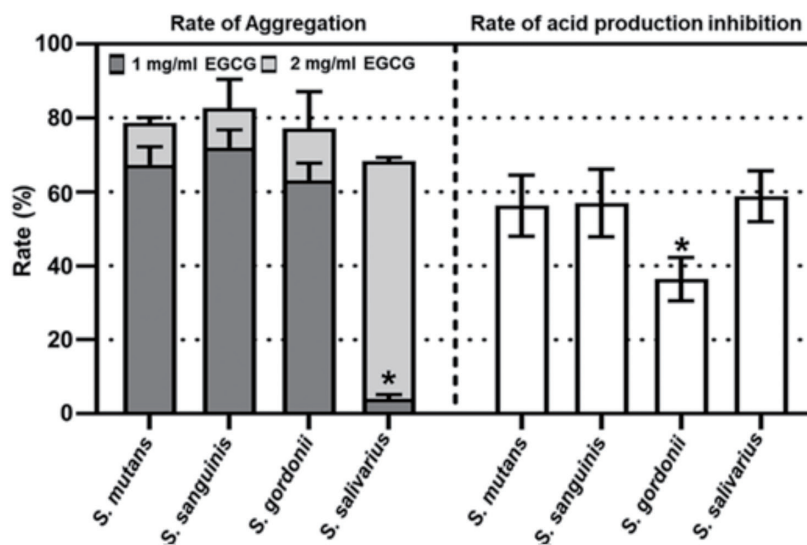


Figure 5. Effects of EGCG on the glucose-induced acid production and aggregation of streptococcus strains

shift the demineralization toward remineralization in the oral ecosystem. Thirdly, EGCG promotes the aggregation of plaque forming-streptococci, which might help to accelerate salivary bacterial removal and reduce bacterial amount. Fourthly, these properties of short-term EGCG treatment were not affected by stimulated saliva, suggesting that EGCG could function in the oral cavity. Although EGCG has multiple antimicrobial properties, it is important for the promotion of oral health that EGCG exerts the essential activities of inhibiting bacterial carbohydrate metabolism (acid production) and promoting bacterial aggregation within a short time in the oral cavity. These findings strongly suggest that the daily use of green tea might be effective for maintaining the healthy oral environment, especially to reduce the bacteria-induced acidification in the oral cavity.

**成果発表：**(予定を含めて口頭発表、学術雑誌など)

Article :

1. Sili Han, Yuki Abiko, Jumpei Washio, Yufang Luo, Linglin Zhang, Nobuhiro Takahashi : Green tea-derived epigallocatechin gallate inhibits acid production and promotes the aggregation of *Streptococcus mutans* and non-mutans streptococci. Caries Research, 2021. (in press)

Oral presentation :

1. Sili Han, Yuki Abiko, Jumpei Washio, Yufang Luo, Linglin Zhang, Nobuhiro Takahashi : The antimicrobial properties of green tea-derived epigallocatechin gallate on *Streptococcus mutans*. The 62th Annual Meeting of Japanese Association for Oral Biology (online), Japan, 2020/09/11-10/09.
2. Sili Han, Yuki Abiko, Jumpei Washio, Yufang Luo, Linglin Zhang, Nobuhiro Takahashi : EGCG inhibits acid production and promotes aggregation of *Streptococcus mutans*. 2020 TU-HKU-FJMU Online Symposium on Oral Health Sciences, Japan, 2020/09/29.

Poster presentation

1. Sili Han, Jumpei Washio, Yuki Abiko, Linglin Zhang, Nobuhiro Takahashi : The antimicrobial properties of green tea derived-catechins on *Streptococcus mutans*. The 99<sup>th</sup> General Session of IADR (International Association of Dental Research), Boston, USA, 2021/07/21-24. (In the preparation)