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Short Communication

Pu'er tea rich in strictinin and catechins prevents biofilm formation of two cariogenic bacteria, *Streptococcus mutans* and *Streptococcus sobrinus*

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Abstract Cariogenic bacteria, such as *Streptococcus mutans* and *Streptococcus sobrinus*, are main pathogens responsible for human dental caries. Pu'er tea is empirically observed to prevent tooth decay. Besides caffeine and catechins commonly found in oolong tea, strictinin is also found as an abundant phenolic compound in Pu'er tea. Infusion of Pu'er tea as well as single compound, strictinin, caffeine or (−)-epigallocatechin gallate (EGCG) was examined for its inhibitory effects on *S. mutans* and *S. sobrinus*. Relatively weak inhibition of bacterial growth was observed for these Pu'er tea constituents. However, biofilm formation of *S. mutans* or *S. sobrinus* was strongly prevented by the infusion of Pu'er tea as well as by strictinin or EGCG, but not caffeine. Relatively, strictinin showed a higher potency than EGCG to prevent biofilm formation. Anti-caries effect of Pu'er tea seems to be resulted from the prevention of biofilm formation of cariogenic bacteria mainly by strictinin and catechins.

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Introduction

Dental caries is one of the most prevalent oral diseases in the world, and nearly half of the world's population suffers this disease.[1] It is caused by the bacteria in the human oral cavity, such as *Streptococcus sanguinis*, *Streptococcus gordonii*, *Streptococcus oralis*, and *Actinomyces* spp. They firstly attach to tooth surface and serve as a bridge between the tooth surface and other oral microorganisms, such as *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus* spp. These microorganisms congregate together on the surface of the tooth and form a sticky biofilm termed dental plaque. The oral flora in the dental plaque corrode the surface of the tooth by hydrolyzing sugars to produce acid, thereby causing tooth decay. Among those oral microorganisms (mostly bacteria), *S. mutans* has been shown to play an important role in the formation of dental caries.[2] Therefore, reducing oral cariogenic bacteria, particularly *S. mutans*, or inhibiting the formation of bacterial plaque biofilm on the tooth surface is considered as an efficient solution to prevent this dental disease.

Anti-caries effects of several chemicals, such as chlorhexidine, fluoride and antibiotics, have been demonstrated in clinical studies; however, undesirable side effects are observed in patients treated with these anti-caries chemicals.[3] Hence, more and more researchers tend to search for natural anti-caries compounds from plant extracts, such as catechol, emetine, quinine, and flavone derivatives.[4] Oolong tea, a popular drink in Taiwan, possesses abundant polyphenolic compounds (mainly catechins), which have been shown to inhibit experimental dental caries in rats infected with *S. mutans* and *S. sobrinus*.[5] Pu'er tea produced in Yunnan, China is another popular drink in Taiwan. Empirically, infusion of Pu'er tea is supposed to be better than that of oolong tea in terms of anti-caries effects for those people using tea infusion as mouth rinse. In this study, three major compounds of Pu'er tea, strictinin, caffeine and the major catechin, (−)-epigallocatechin gallate (EGCG) were tested and compared for their anti-caries activities against *S. mutans* and *S. sobrinus*.

Materials and methods

Two cariogenic bacteria, *S. mutans* (BCRC No.10793) and *S. sobrinus* (BCRC No.14757) were purchased from the Bioresource Collection and Research Center of Food Industry Research and Development Institute (Hsinchu, Taiwan). Pu'er tea was bought from a local tea shop; strictinin was purified from Pu'er tea according to the protocol described previously.[6] Caffeine was bought from Merck KGaA (Darmstadt, Germany), and EGCG were obtained from QualiFlex Co. (Taipei, Taiwan).

S. mutans and *S. sobrinus* were cultured anaerobically (BD GasPak 150 Systems, Oxoid, Thermo Fisher Scientific, Basingstoke, UK) at 37 °C for 24 h on the Tryptic Soy Agar supplemented with 5% sheep blood (Acumedia, Neogen, Lansing, MI, USA). Cell viability of *S. mutans* or *S. sobrinus* in the presence of Pu'er tea infusion (1 or 5 g tea leaves in 100 mL water) or single compound, strictinin, caffeine or EGCG (20, 100 and 500 μM) was examined with the PrestoBlue cell viability reagent (Thermo Fisher, ScientificAvenue, Waltham, MA, USA) at 37 °C for 60 min by detection of fluorescence via 570/610 nm of Excitation/Emission.

Inhibitory effects on the bacterial biofilm formation were analyzed by the crystal violet staining protocol. Cultures of *S. mutans* and *S. sobrinus* were adjusted to 10^7 CFU/mL with the Brain Heart Infusion containing 0.01% sucrose, and loaded into microplate wells (125 μL/well). Bacterial cultures incorporated with Pu'er tea infusion or tea compound were anaerobically incubated at 37 °C for 24 h. Excess medium was firstly decanted, and the bacterial cells in the wells were washed with water twice, air-dried, and then fixed with methanol (125 μL/well) for 15 min. After fixation, methanol was decanted, and the bacterial cells were air-dried and stained with 0.1% crystal violet solution (125 μL/well) at room temperature for 5 min. After decanting the staining agent, the bacterial cells were washed with water three times and destained with 30% acetic acid (150 μL/well). Absorbance at 595 nm was detected by using the Tecan infinite 200 PRO spectrophotometer (Tecan, Mannedorf, Switzerland).

Data were shown as mean values ± standard error of the mean (SEM). One-way analysis of variance and Duncan's post hoc test were used to evaluate significant differences. Statistical calculations were operated by SigmaPlot 12.0 software (Systat, Chicago, IL, USA). Statistically significant was indicated for $p < 0.05$.

Results

Pu'er tea infusion (1 or 5 g/100 mL) was able to reduce the bacterial cell viability, particularly at the high concentration (reduction of viability by approximately 90%), and similar inhibitory effects were observed on the growth of the two bacteria examined (Fig. 1A, B). Three concentrations (20, 100 and 500 μM) of three major constituents, strictinin, caffeine and EGCG were used to evaluate their inhibitory potency on the growth of the two bacteria in the same assay system. The results indicated that rather weak inhibition of bacterial growth was observed for the three Pu'er tea constituents (Fig. 1C, D). No statistically significant inhibition was observed for strictinin, and the reduction of bacterial cell viability by caffeine or EGCG was less than 50% even at the highest concentration (500 μM).

The same two preparations of Pu'er tea infusion were used to examine their prevention on biofilm formation of the two cariogenic bacteria. The results showed that Pu'er tea infusion of either concentration almost completely prevented the biofilm formation of both bacteria (Fig. 1E, F). Furthermore, biofilm formation of either bacterium was significantly prevented by strictinin or EGCG dose-dependently, but not caffeine (Fig. 1G, H). Strictinin of 100 or 500 μM and EGCG of 500 μM were found to strongly prevent the biofilm formation of both bacteria.

Discussion

Tea has been used as mouth rinse to prevent dental caries in Chinese society at least for more than one thousand years. Among various teas commonly consumed in Taiwan,

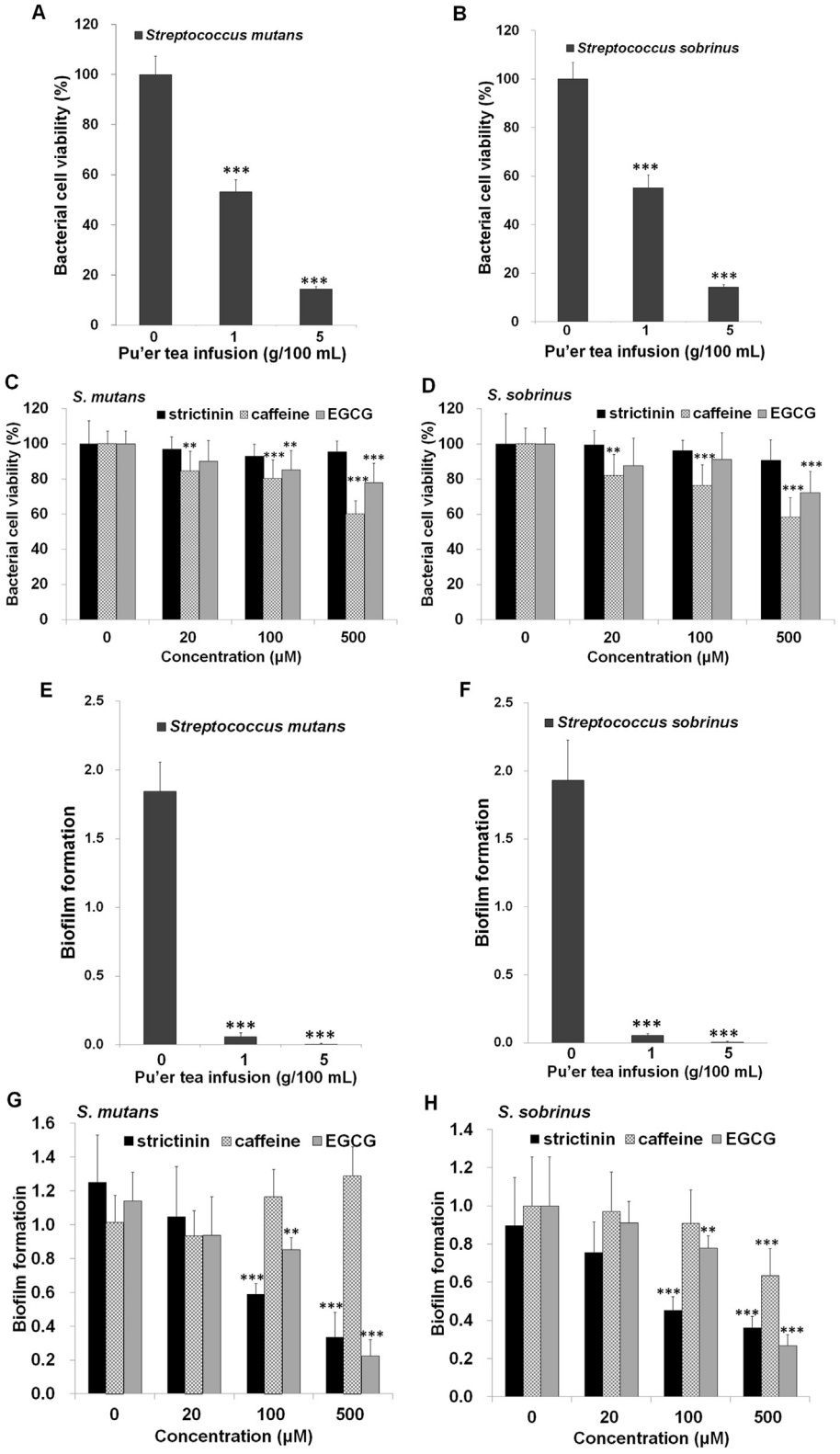

Figure 1 Inhibition on the growth of *Streptococcus mutans* and *Streptococcus sobrinus* and prevention on their biofilm formation by Pu'er tea infusion and three major compounds, strictinin, caffeine and EGCG. Cell viability of *S. mutans* or *S. sobrinus* was examined with the PrestoBlue cell viability reagent. Bacterial biofilm formation was examined by the crystal violet staining. Values are expressed as mean ± SEM (n = 9 per group). Statistically significant symbols, ** and *** indicate *p*-value <0.01 and < 0.001, respectively.

Pu'er tea is empirically regarded as the best tea in terms of anti-caries effects. According to this study, the anti-caries effects of Pu'er tea are proposed to be minorly attributed to its growth inhibition of cariogenic bacteria, such as *S. mutans* and *S. sobrinus*, and majorly resulted from its prevention of bacterial biofilm formation. Furthermore, the anti-caries effects of Pu'er tea are additively contributed by strictinin and catechins. Relatively, strictinin showed a higher potency than EGCG, the major catechin to prevent the biofilm formation of the two cariogenic bacteria. Putatively, the empirical experience for Pu'er tea as the best tea mouth rinse is due to its abundant content of strictinin, which is generally found as a minor constituent in other teas.

In a tea preparation with 10 g of Pu'er tea leaves in 1 L of hot water, approximately 30% of strictinin, caffeine and EGCG contents are released to tea infusion. On average, 10 g of Pu'er tea contains 200—1000 mg of strictinin, 200—500 mg of caffeine, and 200—1000 mg of EGCG.[6] Therefore, the concentrations of strictinin, caffeine and EGCG in the infusion of Pu'er tea are around 100—500, 100—300 and 100—500 μM, respectively. According to this study, the concentrations of strictinin and EGCG in the infusion prepared with Pu'er tea more than 10 g in 1 L of water are higher than 100 μM and thus effective for the prevention of bacterial biofilm formation. It seems that the extract of Pu'er tea can be used as an inexpensive anti-caries natural source for commercialized products.

As side effects are observed in the utilization of synthetic chemicals as anti-caries ingredients, several natural compounds from plant sources with anti-caries activities have been evaluated for their possible replacement of those synthetic compounds. Garlic allicin at a concentration of 600 μg/mL (3700 μM) or higher was found to effectively inhibit *S. mutans* and *S. sobrinus*.[7] Quercetin was shown to inhibit the growth of *S. mutans* in planktonic condition with a concentration higher than 1250 μM, and also found to be effective in the inhibition and reduction of *S. mutans* biofilms at a concentration higher than 16 mg/mL (50,000 μM).[8] Guaijaverin was found to reduce the growth of *S. mutans* with a concentration of 4 mg/mL (10,000 μM).[9] In terms of anti-caries activity, strictinin with an effective concentration lower than 100 μM seems to be much better than allicin, quercetin and guaijaverin examined previously.

Several natural compounds as well as synthetic molecules have been demonstrated to possess anti-caries activity via inhibiting bacterial biofilm formation, and their putative molecular mechanisms are proposed and classified into three categories: sucrose-dependent anti-adhesion, sucrose-independent anti-adhesion and cellular signaling interference.[4] EGCG seems to prevent biofilm formation of *S. mutans* via sucrose-dependent anti-adhesion mechanism as it has been shown to inhibit *S. mutans* glucosyltransferases, which are essential enzymes for the bacterial attachment, biofilm formation and virulence when sucrose is available.[10] It remains to be investigated how strictinin prevents biofilm formation of cariogenic bacteria. Of course, it should not be ruled out that the anti-biofilm formation of strictinin was resulted from its chemical functionality as well as by means of the three biological molecular mechanisms proposed currently. According to its cycling structure, strictinin might be capable of serving as a detergent to solublize or disrupt bacterial biofilms by penetrating or integrating into their chemical structures.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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