Original Article

EFFECT OF POWDERED GREEN TEA MATCHA ON BIOFILM FORMATION BY MUTANS STREPTOCOCCI

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ABSTRACT

Introduction: Antibacterial ingredients with high safety and mild taste that can be used for mouthwash for children are required. Matcha is one of the natural beverages that is made from the leaves of *Camellia sinensis* and widely consumed by Japanese and Asian people. This study aimed to evaluate the effectiveness of matcha in suppressing biofilm formation by mutans streptococci, typical cariogenic bacteria.

Methodology: Five laboratory strains of *Streptococcus mutans* (serotype c, e and f) and *Streptococcus sobrinus* (serotype d and g) were used to evaluate the antibacterial effect of matcha. Matcha extract was added to bacterial cells in Heart Infusion broth supplemented with 1% sucrose (HIS). After incubation for 24 hours, the formed biofilm was dyed by Crystal Violet, and optical density at 490 nm was determined as the amount of biofilm formation. The effect of 0.02% chlorhexidine in HIS and MilliQ in HIS were also measured as well.

Results: The amounts of biofilm formation by all serotypes of mutans streptococci in matcha + HIS were significantly lower than those in MilliQ + HIS as were chlorhexidine in HIS, except *S. sobrinus* serotype g. The differences of amounts biofilm formation by all *S. mutans* (serotype c, e and f) and *S. sobrinus* serotype d in matcha + HIS and in chlorhexidine + HIS were not significant statistically.

Conclusion: Matcha extract has an equivalent effectiveness of 0.02% chlorhexidine against most serotypes of mutans streptococci.

Keywords: *Streptococcus mutans, Streptococcus sobrinus*, chlorhexidine, green tea, mouthwash.

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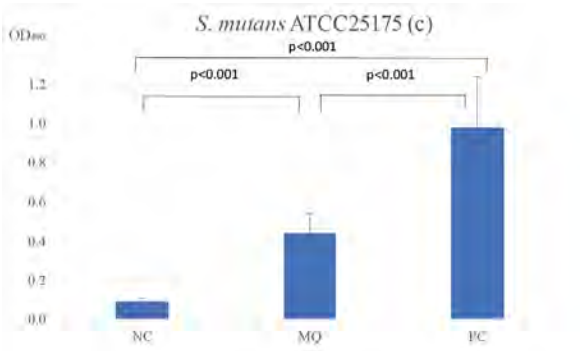
1. Introduction

Efforts to obtain antibacterial ingredients that meet the criteria of comfort, especially taste, are needed to improve the quality of the oral cavity. Oral health is inseparable from the normal flora balance of the oral cavity in order to prevent the occurrence of caries and gingivitis. The use of natural ingredients that are commonly consumed by the wider community in a place needs to be expanded in the functions of maintaining public health, especially oral health [1]. The ease of getting it and the consumption habits of the community makes it easy for materials like this to be accepted by the community if proven to have good effects on oral health. Because caries and gingivitis are also a problem in children in many countries, especially with developmental disabilities, natural ingredients as an antibacterial for the oral cavity are urgently needed to be easily accepted by the child community, both in terms of safety and ease of use [2-4]. Each community has unique ingredients developed in its area, but has not been utilized optimally in addition to regular consumption of food and beverages [5].

Green tea (*Camellia sinensis*) is commercially available in the market in three forms: Loose leaf, bagged and powdered (matcha) [6]. Matcha is generally consumed in Japan as a beverage that has high anti-oxidants such as flavonoids [7]. Many natural foods and beverages that are commonly consumed by people in a region, such as honey in the tropical regions, olive oil, virgin coconut oil and others have been partially known to have antibacterial effects especially in the oral cavity [8]. With many natural sources like ginger and garlic that also have flavonoid and are commonly used in Asia one needs to observe the antibacterial effect on oral bacteria [9]. As a comparison, chlorhexidine has been used for many years as a strong antibacterial standard and is also used in dentistry for preventive measures and treatment but the taste becomes an issue especially for children [10].

The streptococci that are commonly related to the human caries process are *Streptococcus mutans* and *Streptococcus sobrinus*. These cariogenic bacteria are divided into 5 serotypes (c, e, f, d and g) [11]. There has been no study on the antibacterial effects of matcha against each serotype of mutans streptococci. It is expected that the results of research related to antibacterial from natural sources can be used



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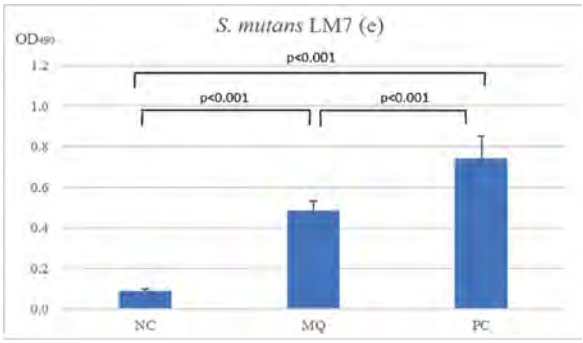

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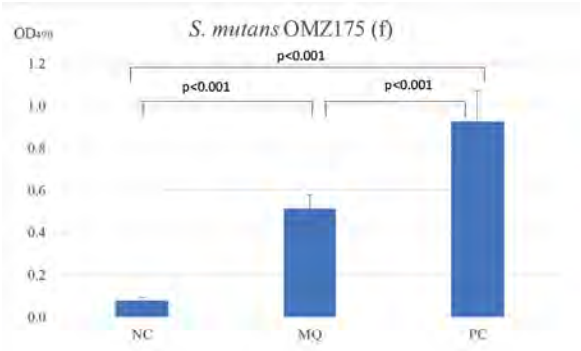

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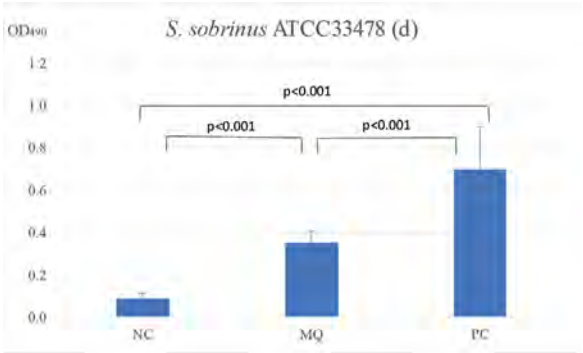

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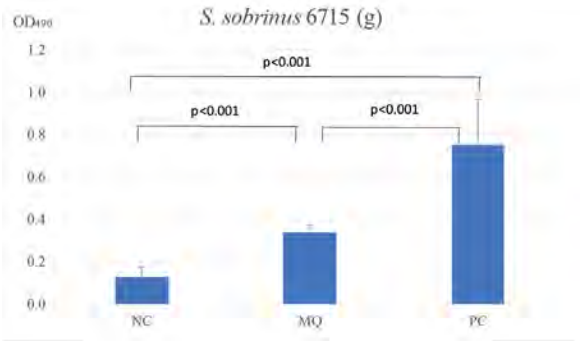

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Figure 1. Biofilm formation in NC (MilliQ alone), MQ (MilliQ + bacterial cells in HIS) and PC (bacterial cells in HIS) by each strain of mutans streptococci:
(a) *S. mutans* ATCC25175 serotype c,
(b) *S. mutans* LM7 serotype e,
(c) *S. mutans* OMZ175 serotype f,
(d) *S. sobrinus* ATCC33478 serotype d,
(e) *S. sobrinus* 6715 serotype g.

to prevent infection in order to minimize chemical substances [12]. This study aimed to evaluate the effectiveness of matcha in suppressing biofilm formation by each serotype of mutans streptococci.

2. Material and Methods

2.1. Preparation of antibacterial solutions

As a matcha solution, 5 gram of powdered green tea matcha (Itoen, Tokyo, Japan) was added to 100 mL of water at 100˚C and boiled for 15 minutes to extract. Prior to use in the laboratory, the solution was filtered using 0.20 µm Minisart filter (Sartorius, Göttingen, Germany). The chlorhexidine solution used in this study was 0.02% and was prepared from 20% chlorhexidine gluconate (Wako Pure Chemical, Osaka, Japan).

2.2. Culture of mutans streptococci

As mutans streptococci, we used 3 laboratory strains of *S. mutans* (serotype c, e, f) and 2 laboratory strain of *S. sobrinus* (serotype d, g) (Table 1). Each strain from -80 ˚C stock was cultured in Brain Heart Infusion (BHI) broth (Becton Dickinson Maryland, USA) at 37 ˚C overnight and then also sub-cultured in BHI broth. The bacterial solution was centrifuged and dissolved in Heart Infusion broth (Becton Dickinson Maryland, USA) supplemented with 1% sucrose (HIS) until it reached 1 x 10^6 CFU/ml which was then used for the biofilm assay as strain culture.

2.3. Biofilm assay

The biofilm formation of each strain was evaluated using a 96 well plate. Each plate was divided into 5 areas of 12 wells and labeled as MT for wells that were filled by matcha solution and strain culture, CHX for wells that were filled by 0.02% chlorhexidine and strain culture, MQ for wells that were filled by MilliQ and strain culture, NC for wells that were filled by milliQ alone as negative control and PC for wells that were filled by HIS and strain culture as positive control (Table 2).
After 24 hours of incubation, the culture supernatant was discarded, and each well was washed twice with 200 µL Phosphate Buffered Saline (PBS). A 200 µL volume of 0.5% crystal violet was then added and incubated at 37 ˚C for 15 minutes.
The remaining crystal violet solution was discarded, the

Table 1. Strains of mutans streptococci used in this study.

Strain	Serotype	
Streptococcus mutans		
ATCC25175	c	(type strain)
LM7	e	
OMZ175	f	
Streptococcus sobrinus		
ATCC33478	d	(type strain)
6715	g	

Table 2. Composition of cultures for biofilm assay.

	Antibacterial solution	Bacterial cells in HIS	HIS	MilliQ	Total
PC	0 µL	100 µL	100 µL	0 µL	200 µL
MT	100 µL	100 µL	0 µL	0 µL	200 µL
CHX	100 µL	100 µL	0 µL	0 µL	200 µL
MQ	0 µL	100 µL	0 µL	100 µL	200 µL
NC	0 µL	0 µL	0 µL	200 µL	200 µL

PC: positive control; MT: matcha solution; CHX: 0.02% chlorhexidine gluconate; MQ: milliQ solution; NC: negative control

biofilm was washed once with 200 mL PBS, and then 200 µL of 96% ethanol was added to each well. The optical density of the biofilm formation was then measured at 490 nm (OD_{490}) using a microplate reader [13].

2.4. Statistical analysis

The OD_{490} values evaluated as biofilm formation were compared between NC, MQ and PC to confirm the validity of the measurement. Then OD_{490} values of MT, CHX, NC and MQ were compared to determine the differences in their effects on biofilm formation. In order to compare the differences, t-test and Bonferoni correction were used.

All statistical analyses were performed using SPSS statistics 19 (IBM, USA); p values of 0.05 or less were considered significant.

3. Results

Fig. 1 shows OD_{490} values which mean amount of biofilm formation of NC (MilliQ alone), MQ (MilliQ + bacterial cells in HIS) and PC (bacterial cells in HIS) by *S. mutans* (serotype c, e and f) and *S. sobrinus* (serotype d and g), respectively. The OD_{490} of PC showed significantly higher values than MQ, and those of MQ showed significantly higher values than NC in all strains (serotype c, e, f, d and g).

Fig. 2 shows OD_{490} values which mean amount of biofilm formation of MT (matcha solution + bacterial cells in HIS), CHX (CHX solution + bacterial cells in HIS), NC (MilliQ alone) and MQ (MilliQ + bacterial cells in HIS), respectively. The OD_{490} of MT, CHX and NC showed significantly lower values than MQ and no significant difference was found between MT, CHX and NC in all *S. mutans* (serotype c, e and f) and *S. sobrinus* (serotype d). In *S. sobrinus* serotype g, the OD_{490} of MT showed lower value than MQ though the difference did not reach statistical significance. The OD_{490} of CHX and NC showed significantly lower values than MQ and MT in *S. sobrinus* serotype g.

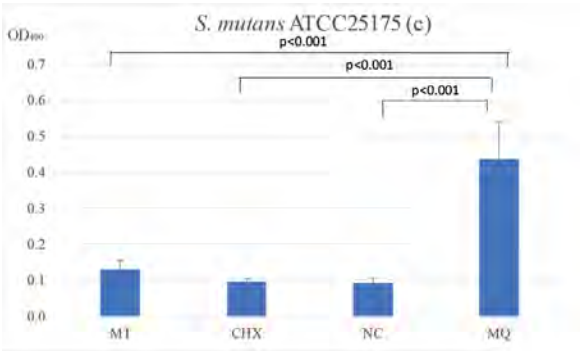
4. Discussion

The OD_{490} of PC showed significantly higher values than MQ and those of MQ showed significantly higher values than NC in all strains, which indicated that the biofilm assay used in this study could evaluate the amount of biofilm formation adequately. The OD_{490} of MT and CHX showed significantly lower values than MQ and the differences between MT, CHX and NC were not significant in all strains except *S. sobrinus* serotype g. This means that the antibacterial effect of matcha solution resemble CHX solution in most strains of mutans streptcocci.

This in-vitro research provides an overview of the antibacterial effects of matcha on various strains of mutans streptococci. Mutans streptococci are considered as typical cariogenic bacteria [14,15]. Mutans streptococci can be classified into five serotypes, with dominant serotypes being slightly different locally. The effect of matcha against *S. sobrinus* serotype g was not significant among all mutans streptococci strains of serotype examined in this study. However, in oral cavity of humans worldwide, *S. sobrinus* serotype g is not a type that found dominantly [16-19]. In other study *S. sobrinus* serotype g more resistance to chlorhexidine than *S. mutans* and *S. sobrinus* serotype d [20]. Therefore, matcha is thought to have the effect of inhibiting biofilm formation for most of mutans streptococci in oral cavity. One of the cariogenic properties of these bacteria owes the high performance of glucan synthesis to sucrose, which contributes to the biofilm formation [21,22]. So, the effectiveness of matcha in suppressing the biofilm formation by mutans streptococci colonization was important to be evaluated in this study. The better antibacterial will suppress more types of strains, meanwhile caries are not only caused by a single bacterial but related to the biofilm activity involving many bacteria [23].

Chlorhexidine is a common ingredient as an antibacterial agent and is widely used for mouthwashes [24-28]. However, chlorhexidine has a taste issue, and it has problems such as side effects on the mucosa and tooth coloring, so it is not suitable for mouthwashes in children [29,30]. The concentration of chlorhexidine used clinically is 0.12% and 0.2%, while in this study using a concentration of 0.02%. It was proved that the concentration of 0.03% was as effective as 0.12% or 0.2% concentration clinically [31-33]. The result of this study revealed that the low concentration of 0.02% chlorhexidine can inhibit biofilm formation of mutans streptococci.

Matcha has long been used for drinking and the safety has been established. The requirements for mouthwash that can be used by children are related to safety issue [34,35]. It is also considered to have less problems with taste. Therefore, it is considered that matcha is suitable as an ingredient for mouthwash for children. The bacteria investigated this time are mutans streptococci and are strongly related to dental caries. However, there are many others bacteria in the oral cavity related to dental caries and other oral disease such as periodontitis. In this study, we did not consider the influence on other such cariogenic bacteria and periodontal disease related bacteria.



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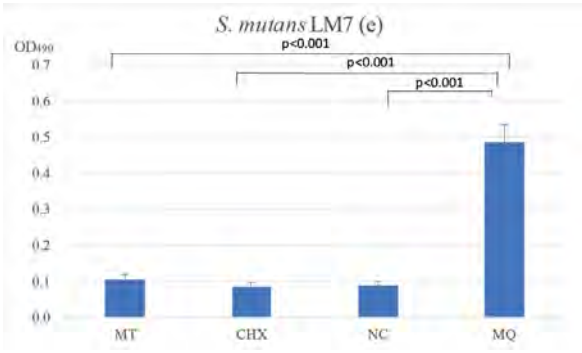

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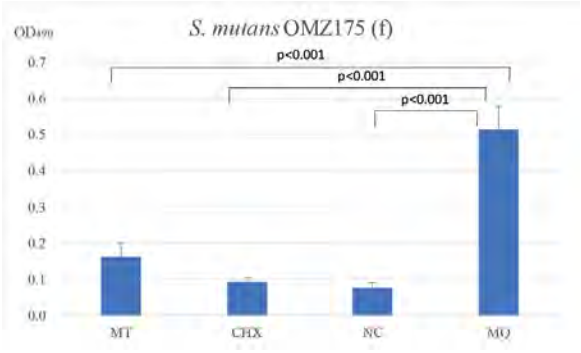

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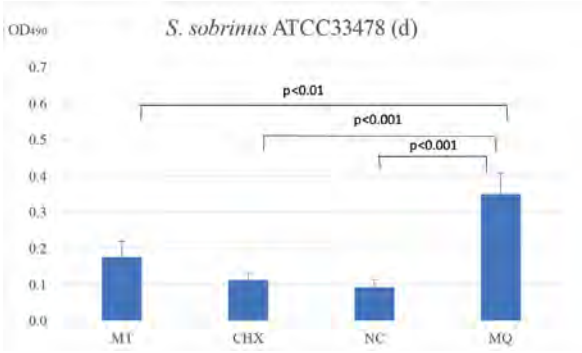

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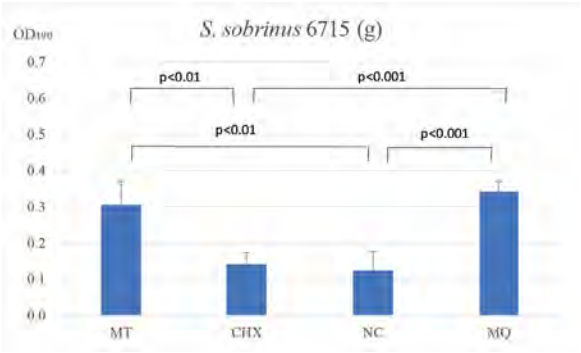

(e)

Figure 2. Effect of matcha extract and chlorhexidine on biofilm formation by each strain of mutans streptococci:
(a) *S. mutans* ATCC25175 serotype c,
(b) *S. mutans* LM7 serotype e,
(c) *S. mutans* OMZ175 serotype f, (
(d) *S. sobrinus* ATCC33478 serotype d,
(e) *S. sobrinus* 6715 serotype g.

Hereafter, it seemed necessary to investigate the antibacterial effect on those bacteria.

5. Conclusion

Matcha has the equivalent effectiveness of 0.02% chlorhexidine against most serotypes of mutans streptococci, namely S. mutans serotype c, e and f and S. sobrinus serotype d. It is suggested that matcha is a promising ingredient as a safe and effective for mouthwashes in children.

Author Contributions

MFR: Idea, experimental design, data analysis, wrote the manuscript. NK: Performed biofilm and spectrophotometry assay, substantial contributed to writing manuscript. HO: Performed substantial contributed to writing manuscript.

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Questions

1. Which was the matcha used in this study?

❑a. 5 g in 100 mL water;
❑b. 0.5 g in 100 mL water;
❑c. 0.05 g in 100 mL water;
❑d. 50 g in 100 mL water.

2. What species of mutans streptococci were used in this study?

❑a. *Streptococcus ferus* and *Streptococcus pneumonia*;
❑b. *Streptococcus mutans* and *Streptococcus sobrinus*;
❑c. *Streptococcus macacae* and *Streptococcus ratti*;
❑d. *Streptococcus criceti* and *Streptococcus downei*.

3. What was the antibacterial used to compare the effectiveness of matcha in this study?

❑a. Ethil alcohol 70%;
❑b. Povidone iodine;
❑c. Chlorhexidine;
❑d. Gentian violet.

4. What was the result of this study?

❑a. All strain of mutans streptococci were suppressed significantly by matcha;
❑b. Only serotype c was suppressed significantly by matcha;
❑c. Only serotype c, e, and d were suppressed significantly by matcha;
❑d. Only serotype c, e, f, and d were suppressed.