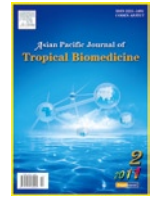




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Fermentation of various sugars and sugar substitutes by oral microorganisms

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ABSTRACT

Objective: To examine acid production of caries-associated strains of oral microorganisms and salivary microorganisms from sugar and sugar substitutes. **Methods:** Standard and clinical strains of *Streptococcus mutans* (*S. mutans*), *Lactobacillus casei* (*L. casei*) and *Candida albicans* were incubated in peptone–yeast–extract media containing 1% test sugar (sucrose, glucose, fructose) or sugar substitutes (xylitol, sorbitol, trehalulose and palatinose) at 37 °C in 5% CO₂ for 24–48 h. The pH of each culture was measured and microbial growth was determined as optical density at 660 nm. Paraffin-stimulated saliva collected from high caries-risk persons were added to media containing 10% test sugar or sugar substitutes. The pH of medium was measured at each time interval from 0–90 minutes. **Results:** All types of sugar and trehalulose could be fermented by all test microorganisms in pH lower than 5.5 except sucrose by standard strain of *L. casei*. All sugar and sugar substitutes supported growth of all organisms except xylitol for *S. mutans*. In the fermentation assay by salivary microorganisms, all sugar could be utilized and produced pH < 5.5 within 10 minutes of incubation and the pH drop was prolonged to until 90 minutes. Conversely, xylitol and palatinose were not fermented by microorganisms in saliva. **Conclusions:** All test microorganisms could ferment sucrose, glucose, fructose and trehalulose to pH lower than 5.5. Sugar alcohols and palatinose were not utilized well by organisms and may be used as sugar substitutes to reduce dental caries incidence. However, further studies particularly clinical investigations are required to evaluate the cariogenicity of these sugar substitutes.

1. Introduction

Dental caries is a major oral health problem found in populations of all age groups. It is initiated by direct demineralization of the enamel of teeth due to acids produced by bacteria. *Streptococcus mutans* (*S. mutans*) and *Lactobacillus* spp. are known by their acidogenic and aciduric properties, more than those of other oral bacteria. They have been shown to have cariogenic potential in both humans and animals^[1]. *Candida albicans* (*C. albicans*), dimorphic fungi, are common colonizers of carious lesions found in children and adolescents. *Candida* can adhere, co-aggregate with oral bacteria and have ability to produce acid from sugar^[2].

The frequency of intake of sugar-containing foods was found to be related to the development of dental caries^[3]. In this regard, sucrose is considered the most cariogenic sugar. It has been reported that the restriction of sucrose

from foodstuffs results in the reduction of dental caries^[4]. One promising way of reducing caries is the substitution of other sweetening substances for sucrose. Trehalulose and palatinose are structural isomers of sucrose commonly used as sucrose substitutes. It has been demonstrated that these sucrose isomers are not utilized by *S. mutans* as substrate to produce acid^[5]. Apart from sucrose isomers, sugar alcohols such as sorbitol and xylitol are used worldwide in a great number of sugar-free products, particularly chewing gums and lozenges, and claimed to have low or non-cariogenic properties^[6].

The purpose of this study was to examine acid production of caries-associated selected strains of oral microorganisms and salivary microorganisms from sugar and sugar substitutes.

2. Materials and methods

2.1 Sugars and sugar substitutes

Sugars used in this study (sucrose, glucose and fructose) and sugar alcohols (xylitol and sorbitol) were purchased from Sigma-Aldrich Co. (USA). And sucrose isomers (trehalulose and palatinose) were purchased from Rajburi

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Sugar Co., Ltd. (Thailand).

2.2. Microorganisms

S. mutans KPSK2, *S. mutans* (clinical isolate), *Lactobacillus casei* (*L. casei*) ATCC 6363, and *L. casei* (clinical isolate) were bacterial strains used in the test. Yeast strains consisted of *C. albicans* ATCC 13802 and *C. albicans* (clinical isolate). All the test microorganisms were obtained from the culture collections of the Oral Microbiology Department, Faculty of Dentistry, Mahidol University.

2.3. Fermentation of microbial strains

All the microbial strains were cultured on Brain Heart Infusion (BHI) agar plates and incubated at 37 °C in 5% CO₂ for 24–48 h. After incubation, the microorganisms were inoculated into 3 mL of peptone–yeast extract (PY media) containing glucose and incubated for 24 h[7]. Then a 20 µL aliquot of each culture was inoculated into 1 mL PY media containing 1% (w/v) test sugar or sugar substitutes and incubated at 37 °C in 5% CO₂ for 24 h. The pH value of each culture was measured using a pH meter (model IQ125, IQ Scientific, USA) and optical density at 660 nm was determined using a spectrophotometer (model C08000, Biowave, UK). An optical density of > 0.5 at 660 nm was considered positive for growth.

2.4. Salivary microorganisms

Paraffin–stimulated saliva were collected from 3 high caries risk persons (aged 20–25 years) who had salivary mutans streptococci > 10⁶ colony forming unit/mL (CFU/mL) and *Lactobacilli* > 10⁴ CFU/mL. These persons were asked to refrain from eating, drinking or having oral hygiene practice for at least 2 h before saliva collection. The saliva were pooled and used further in the fermentation assay.

2.5. Fermentation of salivary microorganisms

A total of 20 µL of pool saliva was added to 1 mL PY media containing 10% (w/v) test sugar or sugar substitutes. The pH of medium was measured at each time interval from 0–90 min.

3. Results

After 24–h incubation, it was found that all of sugars (sucrose, glucose and fructose) could be fermented by the test organisms at pH lower than critical value of 5.5. Sugar alcohol (xylitol and sorbitol) as well as palatinose were not utilized well by all strains since the pH of culture media were not lower than the critical value. This was in contrast to trehalulose, one of sucrose isomers, which could be used as a substrate to produce acids exhibiting a pH drop to below 5.5. When considering the growth, all sugars and sugar substitutes supported the growth of all microorganisms except xylitol for both strains of *S. mutans* (Table 1).

In the fermentation assay by salivary microorganisms, the addition of saliva from high caries risk persons to sugar and sugar substitutes clearly showed that sucrose, glucose and fructose could be utilized well and produced a pH value < 5.5 within 10 min of incubation (Table 2). Furthermore, the pH drop was prolonged to until 90 min. Fermentation of trehalulose to a critical pH was observed after 20 min. Conversely, xylitol and palatinose were not fermented well by microorganisms in saliva. In the case of sorbitol, though the pH drop was not lower than critical value, a pH of 5.5 was detected at 30 min.

4. Discussion

The production of organic acids from dietary sugars is a key factor in the caries process. Sucrose is easily produced

Table 1

Fermentation of sugar and sugar substitutes by oral microorganisms.

strains	culture pH and growth						
	Sucrose	Glucose	Fructose	Xylitol	Sorbitol	Trehalulose	Palatinose
<i>S. mutans</i> KPSK2	4.8 + ^{a,b}	4.5 +	4.8 +	6.7 –	6.0 +	4.9+	6.0 +
<i>S. mutans</i> (clinical isolate)	5.0 +	4.7 +	4.9 +	6.8 –	5.7+	5.0 +	6.5 +
<i>L. casei</i> ATCC6363	5.4 +	4.7 +	5.0 +	6.2 +	6.4 +	5.0 +	6.5 +
<i>L. casei</i> (clinical isolate)	5.4 +	4.5 +	5.2 +	6.0 +	6.2 +	5.2 +	6.2 +
<i>C. albicans</i> ATCC13801	5.4 +	5.0 +	5.3 +	7.1 +	6.9 +	5.3 +	7.0 +
<i>C. albicans</i> (clinical isolate)	5.3 +	5.3 +	5.0 +	6.9 +	6.5 +	5.0 +	6.5 +

^a Mean pH values were obtained from three independent experiments. (Remark: critical pH of demineralization = 5.5);

^b Positive growth was indicated by optical density of more than 0.5 at 660 nm.

Table 2

Fermentation of sugar and sugar substitutes by salivary microorganisms.

Time (min)	culture pH						
	Sucrose	Glucose	Fructose	Xylitol	Sorbitol	Trehalulose	Palatinose
0	7.0	7.0	7.1	7.1	7.1	7.0	7.1
10	5.4	5.4	5.5	6.4	6.5	5.6	7.2
20	4.8	5.0	5.4	6.5	5.8	4.7	6.4
30	4.2	5.0	4.5	6.7	5.5	4.3	6.5
40	4.0	4.3	4.6	7.1	6.3	4.0	7.0
60	3.6	4.2	4.5	7.0	6.5	4.0	6.9
90	4.8	5.3	5.4	6.9	6.6	5.9	7.1

Data are mean pH values obtained from three independent experiments. (Remark: critical pH of demineralization = 5.5)

from sugar cane or sugar beet and contributes highly to diets and processed foods as a sweetener, preservative or texture modifier. A broad consensus exists that the amount and frequency of consumption of sucrose-containing foods have significance on caries incidence. Moreover, using animals as study models to assess the caries potential of sugars revealed that sucrose gave the highest caries scores compared with other fermentable sugars[7]. Its molecule consists of a glucose unit joined to a fructose unit by α -D-glucosidic linkage. The result obtained from the present study was in agreement with previous studies that sucrose, glucose and fructose could be fermented by caries-associated microorganisms and microorganisms in saliva producing acids lowering pH to below the critical value. Therefore, the replacement of dietary sucrose or sugar with sweeteners that cannot be metabolized or that are metabolized slowly by oral microorganisms will prevent enamel demineralization. Sucrose or sugar substitutes can play an important role in shifting the caries process in favor of maintaining dental health, and they should be recommended as part of an overall preventive treatment plan for patients at high risk of developing caries.

Sugar alcohols or polyols are classified as non-fermentable sugars. The most common polyols are xylitol and sorbitol and have been used extensively as sugar substitutes for oral health benefit. Our result showed that all of the test microorganisms could not ferment xylitol or sorbitol to generate a pH lower than critical value even though pH value of 5.5 could be observed when sorbitol was utilized by salivary microorganisms from high caries risk persons at 30 min. In this study, the value of 5.5 was used as a critical threshold for inducing caries since demineralization of tooth surface was observed at this pH[8]. Previous studies have shown that xylitol possess anti-caries properties due to its ability to inhibit growth of mutans streptococci, interfere with glycolysis and is not fermented by oral microorganisms[9]. For sorbitol, even though most microorganisms dominating in dental plaque cannot utilize sorbitol as their energy source, *S. mutans* and some species of *Lactobacilli* do ferment sorbitol slowly[10,11]. This is in accordance with results found in the present study.

Palatinose and trehalulose are sucrose derivatives industrially produced by enzymatic conversion of the glucosyl linkage of sucrose. Form α -glucosyl-(1-2)- β -fructoside, α -glucosyl-(1-6)-fructose and α -glucosyl-(1-1)-fructose are formed. It is known that these sugars are hydrolyzed to glucose and fructose by disaccharidase in the small intestine and their sweetness levels are about half of sucrose. According to our result, palatinose was not utilized well by all microbial strains and salivary microorganisms since acid production in culture media did not lower the pH to below critical value. It has been reported that no acid production from palatinose could be detected in experiments involving animal caries infected with mutans streptococci[12]. However, in humans, the production of acid was found to vary among individuals[13]. In contrast to palatinose, trehalulose could be used as a substrate by the test strains and exhibited a pH drop to below critical value after 20 min of incubation. Matsuyama *et al*[5] demonstrated that predominant bacteria isolated from human dental plaque were able to ferment trehalulose lowering the pH to below 5.5. Another study in mutans streptococci revealed that trehalulose was a weaker and slower acid fermenter compared with sucrose[14].

In conclusion, the results from this observation

suggest that all of test sugars could be fermented by oral microorganisms to a pH of tooth demineralization and sugar alcohols as well as palatinose may be promising substitutes for sugar to reduce incidence of dental caries. However, a conclusive evaluation concerning the cariogenicity of these sugar substitutes cannot be made only from the present findings. Further studies particularly clinical investigations are required.

Conflict of interest statement

We declare that we have no conflict of interest.

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