Acid production from Lycasin®, maltitol, sorbitol and xylitol by oral streptococci and lactobacilli

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Edwardsson, S., Birkhed, D. & Mejàre, B. Acid production from Lycasin®, maltitol, sorbitol and xylitol and by oral streptococci and lactobacilli, *Acta Odont. Scand.* 35, 257–263

The acid production from maltitol was compared with the acid production from hydrogenated starch hydrolysate (Lycasin®), sorbitol and xylitol by a number of oral strains and reference strains of *Streptococcus mutans, S. sanguis, S. salivarius, S. mitior, S. milleri, S. faecalis, S. faecium, S. avium, Lactobacillus casei* and *L. salivarius*. The polyols were added to a final concentration of 1.0% to two different basal media. Incubation was performed at 37°C for 7 days after which the pH was recorded. Maltitol was fermented only by the lactobacilli (about two thirds of the strains). Lycasin® was fermented by all strains of *S. faecalis*, more than 90% of the lactobacilli, about half of the *S. sanguis* strains, about one third of the *S. mutans* strains, and by a few other streptococcal strains. Acid production from sorbitol was observed among more than 80% of the *S. mutans* strains and the *S. faecalis* strains and most of the lactobacilli strains. Sorbitol-fermenting strains of *S. sanguis* and of *S. mitior*, all isolated from sorbitol-consumers, were observed. No other sorbitol-fermenting streptococci were found. Only the reference strains *L. salivarius subsp. salivarius* ATCC 11741 and *S. avium* ATCC 14025 fermented xylitol.

Key-words: Microbiology; sugar alcohols

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Recently great interest has been focused on various sucrose substitutes in sweets (*Mühlemann*, 1966; *Newbrun*, 1973; *Scheinin*, 1973). This interest has included several investigations on sugar alcohols based on the fact that these polyols cause little alterations of the pH in dental plaque *in situ* (*Frostell*, 1965; 1973; *Frostell et al.*, 1974; *Mühlemann & de Boever*, 1969). Sorbitol and hydrogenated starch hydrolysate (Lycasin®) are already used in various kinds of sweets and beverages. In addition, xylitol, the sugar substitute of the «Turku sugar studies» (*Scheinin & Mäkinen*, 1975), has now become incorporated in commercially available pastilles and chewing-gum. Maltitol has been developed in Japan and is used there as a food ingredient (*Naito*, 1971).

Gehring (1971) showed that strains of the species *S. mutans*, known to be caries-inducing in experimental animal system, were capable of degrading Lycasin© and

Received for publication, April 24, 1977

sorbitol to acid at a low rate in comparison with the acid production from sucrose. Slow acid production from Lycasin® in comparison with glucose has been observed by *Bramstedt & Trautner* (1971), who studied one caries-inducing and one non-caries-inducing streptococcal strain.

Sorbitol is fermented *in vitro* by a significant number of strains of *S. mutans* (*Carlsson*, 1968; *Edwardsson*, 1968). Most enterococci also ferment sorbitol (*Deibel* and *Seeley*, 1974). *Carlsson* (1968) and *Mejàre & Edwardsson* (1975) observed that some strains within *S. sanguis* and *S. mitior* were capable of fermenting sorbitol. *L. casei* and *L. salivarius*, which are normal inhabitants in the human oral cavity, also produce acid from sorbitol (*Rogosa*, 1974).

Present knowledge points to a prevalence of few xylitol-fermenting microorganisms in the human oral cavity. *Gehring* (1971) and *Gehring et al.* (1975) found no oral streptococci with this property. No adaptation or mutation of the dental plaque microbiota to degrade xylitol with acid production has been reported (*Knuuttila & Mäkinen*, 1975). The only observation on xylitol-fermentors, which may be present in the human mouth, is the report by *Rogosa* (1974) that *L. salivarius subsp. salivarius* is capable of producing acid from this polyol. *S. avium*, serotype Q, which has been isolated from the human faeces, is one of the few species within *Streptococcus*, which can produce acid from xylitol (*Gehring*, 1971; *Deibel & Seeley*, 1974).

Little information is available about the capacity of oral bacteria to ferment maltitol. *Naito* (1971) reported a study including four streptococcal strains and two lactobacillus strains. *Ikeda et al.* (1973) studied nine streptococci, representing six species, including the species *S. sanguis*, *S. mutans*, *S. salivarius* and *S. mitis*. Both reports concluded that the studied strains fermented maltitol only slightly or not at all.

The aim of the present study was to compare the acid production from maltitol with that from Lycasin®, sorbitol and xylitol by strains of oral microorganisms representing the genera *Streptococcus* and *Lactobacillus*.

MATERIAL AND METHODS

Bacterial strains

The strains selected for the study and their sources are given in Table I. All available sorbitol-fermenting strains of oral streptococci were included in the study. Most of the strains had been stored in the lyophilized state, while some recently isolated strains were maintained on blood agar or in VMG III (*Möller*, 1966). During the investigation all strains were kept on blood agar in a refrigerator (6°C) and subcultured every third week.

Basal media

The strains were tested in the following basal media:

(1) All strains were studied in peptone yeast extract basal medium (PY-broth) under complete anaerobic conditions (PRAS-technique) according to *Holdeman & Moore* (1972). pH was adjusted to 6.9 When the lactobacilli were tested the pH was adjusted to 6.6.
(2) The streptococcal strains were also studied in streptococcus broth (Sb-broth) (*Jordan, Fitzgerald & Bowler*, 1960) without glucose. pH was adjusted to 7.0.
(3) The lactobacilli were also studied in MRS-broth (*de Man, Rogosa & Sharpe*, 1960). pH was adjusted to 6.4.

Table I. *Strains included in the study and their sources*

Strains	Origin
STREPTOCOCCI	
S. avium	ATCC[1] 14025
S. faecalis, 26 strains	Isolated from human gingival crevice or infected dental root canals
S. faecium, 4 strains	Isolated from human infected dental root canals
S. milleri	NCTC[2] 10708
S. MG	ATCC 9895
S. milleri, 15 strains	Isolated from human dental plaque, gingival crevice, tongue, saliva or carious dentine
S. mitior	NCTC 10712
S. mitior, 13 strains	Isolated from human dental plaque, tongue, saliva or carious dentine
S. viridans	NCTC 10449
S. mutans, 14 strains	Isolated from human dental plaque
S. salivarius	ATCC 9759
S. salivarius	ATCC 13419
S. salivarius	NCTC 8618
S. salivarius, 12 strains	Isolated from human tongue, saliva or dental root canal cultures
S. sanguis	ATCC 10556
S. sanguis	ATCC 10557
S. sanguis	ATCC 10558
S. sanguis, strains 804	*Carlsson* (1968)
S. sanguis, strain 903	*Carlsson* (1968)
S. sanguis, 21 strains	Isolated from human dental plaque, saliva, carious dentine or infected dental root canals
LACTOBACILLI	
L. casei subsp. casei	NCTC 151
L. casei subsp. rhamnosus	ATCC 7469
L. fermentum	NCTC 1750
L. salivarius subsp. salivarius	ATCC 11741
L. salivarius subsp. salicinius	NCDO[3] 929
L. casei subsp. rhamnosus, 37 strains	Isolated from human carious dentine
L. salivarius subsp. salicinius, 1 strain	Isolated from human carious dentine

[1] American Type Culture Collection, Bethesda, Maryland, U.S.A.
[2] National Collection of Type Culture, London, England
[3] National Collection of Dairy Organisms, Shinfield, Nr Reading Berkshire, England

Addition of carbohydrates to basal media

To the three basal media carbohydrates were added to a final concentration of 1% (w/v). The following carbohydrates were tested: glucose (analytical reagent, Mallinckrodt Chemical Works, St. Louis, U.S.A.), Lycasin® (standard candy quality, Lyckeby Stärkelseförädling AB, Lyckeby, Sweden), sorbitol (extra pure, Merck, Darmstadt, W. Germany), maltitol (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, supplied by A/S Benzon, Copenhagen, Denmark) and xylitol (Oy Finnsugar Trading Ltd., Helsinki, Finland). According to the manufacturer's declaration the maltitol product used contained maltitol 93.5%, maltotriitol 3.0%, sorbitol 2.3%, higher sugar alcohols 1.1% and reducing sugar 0.1%. The carbohydrates were added to the basal broths before sterilization. The PRAS-substrates and MRS-broth were autoclaved at 120°C for 15 min and the streptococcus broth at 115°C.

Inoculum

For the streptococcal strains the inoculum consisted of two drops (approximately 0.1 ml) of an 18-hrs culture in streptococcus broth (*Jordan et al.*, 1960) containing 0.4% (w/v) of glucose and 0.05% (v/v) of Tween 80. For the lactobacilli the inoculum consisted of two drops (approximately 0.1.ml) of an 18-hrs culture in MRS-broth (*de Man et al.*, 1960) containing 1% (w/v) of glucose. When the number of viable cells was less than 10^4 in the inoculum the test was discarded.

Incubation and pH-determination

Incubation was performed at 37°C for 7 days and the turbidity was noted by eye and the pH was recorded with a pH-meter (pH-meter 28, ⅘ Radiometer, Copenhagen, Denmark). For the streptococcal strains the results were recorded as «positive» when the pH was ≦ 5.5, «weak positive» 5.6 – 5.9 and as «negative» (not-fermenting) ≧ 6.0. The results for the lactobacilli were recorded as «positive» when the pH was ≦ 5.0, «weak positive» 5.1 – 5.5 and «negative» (not-fermenting) ≧ 5.6.

The tests were run in duplicate. Where the results did not agree a third test was performed. The final result was recorded as that, which occurred twice in three tests.

RESULTS

All the strains fermented glucose and the final pH was always less than 5.5 for the streptococci and 5.0 for the lactobacilli.

The results for all the strains with Lycasin®, maltitol and sorbitol are given in Table II. The only two strains which fermented xylitol were the reference strains *S. avium* ATCC 14025 and *L. salivarius subsp. salivarius* ATCC 11741 (Table II).

DISCUSSION

The present study was performed to compare the acid production from maltitol with that from Lycasin®, sorbitol and xylitol by various oral streptococci and lactobacilli. The method does not permit any conclusions about the rate of acid production.

No streptococcal strain fermented maltitol, which agrees with findings by *Naito* (1971) and *Ikeda et al.* (1973). In contrast to *Naito* (1971), who only studied one strain of *L. fermenti,* several lactobacilli of the common oral species *L. casei* and *L. salivarius* (*Rogosa et al,* 1953) were included in the present study. Most of these strains fermented maltitol. However, the lactobacilli make up only a small part of the regular oral plaque flora (*Socransky & Manganiello,* 1971). The results therefore do not indicate that maltitol is fermented to any great extent in dental plaque. This view is supported by the results from mouth rinsings with a 50% water solution of maltitol in 22 persons (*Birkhed, Ahldén & Frostell,* in preparation).

Gehring (1973) studied the acid production from isomaltitol and isomaltulose with strains of the species *S. mutans, S. sanguis* and *S. salivarius* and found only slow fermentation. However, these compounds, chemically related to maltitol, may be metabolized in different ways in these microorganisms.

Lycasin® is a mixture of free sorbitol, maltitol and higher hydrogenated saccharides and dextrines (*Frostell,* 1971). It is therefore probable that the product can be decomposed by various enzymes, such as sorbitol-6-phosphate dehydrogenase and alpha-amylase. It is possible that a microorganism must produce both these enzymes and perhaps others in order to be able to ferment Lycasin® effectively. Several of the strains which fermented sorbitol could also produce acid from Lycasin®. In this study lactobacilli, *S. faecalis,* several strains of *S. sanguis* and about one third of *S. mutans* strains had this property. However, the decrease in pH of human dental plaque at contact with Lycasin®, which has been observed by *Frostell* (1971; 1973) and by *Mühlemann & de Boever* (1969), might only be partly explained as due to fermentation by the studied bacterial groups. Other oral microorganisms presumably exist which also can attack Lycasin®. Moreover, the salivary alpha-amylase may to some extent be

Table II. *Acid production from Lycasin®, maltitol, sorbitol and xylitol by oral streptococci and lactobacilli*

Species	Total number of strains	Broth	Number of strains and final pH after 7 days incubation in broth containing[1]:								
			Lycasin®			Maltitol			Sorbitol		
			⩽5.5	5.6–5.9	⩾6.0	⩽5.5	5.6–5.9	⩾6.0	⩽5.5	5.6–5.9	⩾6.0
S. avium	1	PY	0	1	0	0	0	1	1	0	0
		Sb	1	0	0	0	0	1	1	0	0
S. faecalis	26	PY	23	3	0	0	0	26	26	0	0
		Sb	26	0	0	0	0	26	26	0	0
S. faecium	4	PY	0	4	0	0	0	4	0	0	4
		Sb	4	0	0	0	0	4	0	0	4
S. milleri	17	PY	0	0	17	0	0	17	0	0	17
		Sb	1	1	15	0	0	17	0	0	17
S. mitior	14	PY	0	3	11	0	0	14	4	1	9
		Sb	0	3	11	0	0	14	3	2	9
S. mutans	15	PY	0	0	15	0	0	15	13	0	2
		Sb	0	5	10	0	0	15	13	0	2
S. salivarius	15	PY	0	0	15	0	0	15	0	0	15
		Sb	0	0	15	0	0	15	0	0	15
S. sanguis	26	PY	1	11	14	0	0	26	12	0	14
		Sb	10	4	12	0	0	26	12	0	14
			⩽5.0	*5.1–5.5*	*⩾5.6*	*⩽5.0*	*5.1–5.5*	*⩽5.6*	*⩽5.0*	*5.1–5.5*	*⩾5.6*
L. casei	39	PY	15	7	17	27	3	9	38	0	1
		MRS	33	5	1	30	1	8	38	0	1
L. fermentum	*1*	*PY*	*0*	0	1	0	0	1	0	0	1
		MRS	0	0	1	0	0	1	0	0	1
L. salivarius	3	PY	1	0	2	0	0	3	3	0	0
		MRS	3	0	0	0	0	3	3	0	0

[1] *S. avium* ATCC 14025 in PY with xylitol pH 5.4 and in Sb with xylitol pH 5.2. *L. salivarius subsp. salivarius* ATCC 11741 in PY with xylitol pH 4.2 and MRS with xylitol 4.7. All other strains were negative with xylitol.

involved in the Lycasin® degradat. ıe human oral cavity.

The fermantability of sorbitol by *S. mutans* and lactobacilli is in agreement with earlier investigations (*Grubb*, 1945; *Crowley et al.*, 1956; *Gehring*, 1973; *Rogosa*, 1974). In the present study, some strains of *S. sanguis* and *S. mitior* also showed the ability to ferment sorbitol (Table II). These strains had been isolated from subjects, who consumed sorbitol-containing chewing-gums or sweets for a long period of time. The findings indicate that sorbitol-fermenting strains of *S. sanguis* and *S. mitior* can be found in dental plaque of subjects with such habits.

Apart from *L. salivarius subsp. salivarius* ATCC 11741, no oral strains of streptococci or lactobacilli fermented xylitol. This is in agreement with *Gehring* (1971) and *Gehring et al.* (1975). *Birkhed et al.* (in preparation) were also unable to reveal any pH-decrease after mouth rinsings with 50% water solutions of xylitol in 22 persons and *Scheinin, Mäkinen & Ylitalo* (1975) have observed an unusually low caries activity when all sucrose in the diet was substituted by xylitol.

The present study indicates that maltitol, from a bacteriological point of view, may be an alternative to the other sugar substitutes. However, it should be pointed out that the study only demonstrates the *in vitro* situation.

Acknowledgements. We wish to thank Professor *Göran Frostell* and Professor *Jason M. Tanzer* for valuable discussions. The investigation was supported by A/S Alfred Benzon, Copenhagen, Denmark.

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