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ORIGINAL ARTICLE

# Fermentation of sugars and sugar alcohols by plaque *Lactobacillus* strains

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**Abstract**
*Objective* The objective was to analyse the ability of *Lactobacillus* strains isolated from supragingival plaque of subjects with hyposalivation and from healthy controls to ferment sugars and sugar alcohols.
*Material and methods* Fifty strains isolated from interproximal plaque from subjects with radiation-induced hyposalivation (25 strains), subjects with primary Sjögren's syndrome (16 strains) and from subjects with normal salivary secretion rate (9 strains) were tested. Growth and pH were determined after 24 and 48 h of anaerobic incubation in vials containing basal media with 1 % of glucose, fructose, sucrose, mannitol, sorbitol or xylitol.
*Results* No differences between strains isolated from hyposalivated subjects and controls were detected. All strains lowered the pH to <5.0 from fructose and the majority of the strains from glucose and sucrose. A pH of <5.5 was seen for 52 % of the strains using mannitol, 50 % using sorbitol and 36 % using xylitol. The ability to produce acids from sugars and sugar alcohols was highest among strains of *Lactobacillus rhamnosus*, *Lactobacillus casei* and *Lactobacillus paracasei* and lowest among *Lactobacillus fermentum* strains.
*Conclusion* A large number of *Lactobacillus* strains are able to ferment not only sugars but also the sugar substitutes mannitol, sorbitol and xylitol to pH levels critical for enamel demineralisation.
*Clinical relevance* Our findings suggest that products containing mannitol, sorbitol and/or xylitol may contribute to the acidogenic potential of the dental plaque and especially in hyposalivated subjects with high numbers of lactobacilli.

**Keywords** Fermentation · Hyposalivation · *Lactobacillus* · pH · Sorbitol · Xylitol

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## Introduction

Subjects with hyposalivation often harbour high numbers of *Lactobacillus* spp. both in saliva and in supragingival plaque [1–6]. In a previous study, the prevalence of different *Lactobacillus* species in the supragingival plaque of subjects with hyposalivation due to primary Sjögren's syndrome or radiation therapy in the head and neck region and in controls with normal salivary secretion rates was analysed [7]. *Lactobacillus fermentum* and *Lactobacillus casei* were the most prevalent species in anterior plaque whereas *Lactobacillus rhamnosus* and *L. fermentum* were the most prevalent in posterior plaque. Subjects with high *Lactobacillus* counts had a more acidogenic plaque than those with no or low numbers of lactobacilli in their plaque [7].

*Lactobacillus* spp. are able to ferment a wide range of carbohydrates resulting in acid production [8]. Strains of *Lactobacillus plantarum* and *Lactobacillus salivarius* have been found to ferment the sugar substitutes sorbitol and xylitol leading to a *pH*<5.5 [9], considered as the critical level for enamel demineralisation. It has also been shown that *Lactobacillus* strains can adapt to xylitol fermentation [10]. Sorbitol and xylitol are common sugar substitutes in toothpaste and other fluoride-containing products, such as chewing gums and saliva-stimulating products, which are

frequently used by subjects with hyposalivation [2]. Therefore, especially in subjects with hyposalivation, it is possible that lactobacilli are favoured by frequent access to sugar substitutes and can adapt to fermentation of these, giving the lactobacilli an advantage to other acidogenic microorganisms. Little is, however, known about the variation in ability of different species and strains of lactobacilli to ferment carbohydrates and sugar alcohols.

Our hypothesis was that *Lactobacillus* strains able to ferment the sugar substitutes mannitol, sorbitol and xylitol are frequently found in the supragingival plaque in subjects with hyposalivation due to primary Sjögren's syndrome (pSS) and subjects with radiation-induced hyposalivation (RT).

The aim of the present study was to analyse the fermentation patterns of *Lactobacillus* strains isolated from supragingival plaque from subjects with pSS, subjects with radiation-induced hyposalivation (RT) and controls with normal salivary secretion rate.

## Materials and methods

### Supragingival plaque and *Lactobacillus* spp

The *Lactobacillus* strains included in the present study were isolated from supragingival plaque of anterior and posterior tooth surfaces from six RT subjects, three pSS subjects and from five healthy controls. Data from these subjects regarding general health, medication, salivary secretion rate and microflora [3, 7, 11] as well as the methods for plaque sampling and isolation and identification of *Lactobacillus* spp. have previously been presented [7].

Briefly, after refraining from interproximal tooth cleaning for 3 days, plaque was collected with sterile toothpicks from one upper anterior and one upper posterior interproximal area. After dilution and inoculation on Rogosa agar plates for 48 h, lactobacilli were randomly selected using a template with three circles representing 10 % of the agar surface area, and all colonies within the circles were recultivated and saved [7]. Lactobacilli from six RT subjects (59 isolates), three pSS subjects (40 isolates) and five controls (11 isolates) were isolated and saved. The 110 isolates were further identified using polymerase chain reaction and restriction fragment length polymorphism.

### *Lactobacillus* isolates for fermentation tests

Out of the 110 *Lactobacillus* isolates, 66 were selected for fermentation tests (22 from pSS subjects, 33 from RT subjects and 11 from controls). For the hyposalivated subjects, all isolates from plaque samples from which one to two isolates of *Lactobacillus* spp. had been collected and about 50 % of the isolates collected from plaque with ≥3 isolates, were included in the test. Isolates of the same species giving similar fermentation patterns (pH values varying between 0 and 0.5 pH units) were presumed to represent the same strain and were counted together. From three pSS, four RT and two control plaque samples respectively, two isolates were counted together, and from respectively one pSS and one RT plaque samples, four isolates were counted together. Fifty isolates (25 from RT subjects, 16 from pSS subjects and 9 from controls) of various species and fermentation patterns are therefore reported here (Table 1). Most of the isolates belonged to the species *L. fermentum*, *L. casei*, *L. rhamnosus* and *Lactobacillus paracasei* or were unidentified.

### Fermentation tests

*Lactobacillus* isolates were transferred from Cryobank tubes to Rogosa agar plates, which were incubated in 90 % $CO_2$ and 10 %$N_2$ at 36 °C for 48 h. A representative colony was transferred to a vial with 5.0 ml of basal media consisting of 5 g/l Thiotone peptone (BBL Microbiology Systems, Cockeysville, MD, USA), 5 g/l Trypticase peptone (BBL), 5 g/l yeast extract (Becton, Dickinson and Company, Franklin lakes, NJ, USA), 20 ml salt solution (pH 7.4) and 1 % glucose (Difco, Detroit, MI, USA). The vial was incubated anaerobically until exponential growth phase, and its optical density (i.e an optical density of 0.4–0.6) was determined with a spectrophotometer (Novaspec II Spectrophotometer, Pharmacia Biotech) at a wavelength of 480 nm. Hundred microlitres of the bacterial suspension was transferred to vials with 5.0 ml basal media with 1 % of glucose (Merck, Germany), fructose (Merck), sucrose (Difco), mannitol (Difco), sorbitol (Merck) or xylitol (kind gift from AB R. Lundberg, Sweden). The optical density (480 nm) and pH (pH meter, Metrohm 632) of the solution were determined after 24 and 48 h of anaerobic incubation.

### Statistical methods

The fermentation tests were performed twice for all isolates and a mean value was calculated. In the cases where the pH differed more than 10 % between the two tests, the fermentation test was performed the third time and a mean was calculated from the three values. The mean value was used for the isolates (two to four) of the same species giving similar fermentation patterns and counted together. Differences in pH after sugar fermentation between lactobacilli isolated from RT, pSS and controls, between lactobacilli isolated from anterior and posterior dental plaque and between different species of lactobacilli were analysed using ANOVA. When the ANOVA rejected the multi-sample hypothesis of equal means, multiple comparison testing was

**Table 1** Group and number of isolates subjected to fermentation tests

| Group | Isolates selected for fermentation tests |
|---|---|
| pSS ($n$=16) | *L. fermentum* (5), *L. casei* (3), *L. rhamnosus* (3), *L. paracasei* (2), *L. acidophilus* (1), *L. gasseri* (1), unidentified (1) |
| RT ($n$=25) | *L. fermentum* ( 5), *L. rhamnosus* (5), *L. casei* (5), *L. paracasei* (3), *L. salivarius* (2), unidentified (5) |
| Controls ($n$=9) | *L. fermentum* (2), *L. paracasei* ( 2), *L. casei* (2), unidentified (3) |

performed with Fisher's exact test. Testing was performed two-tailed and at the 1 % level.

## Results

There were no significant differences in growth and acid formation between *Lactobacillus* isolates from anterior and posterior dental plaque. Neither were there any significant differences between species isolated from the different patient groups. Furthermore, the number of isolates from controls was low and therefore all isolates of different *Lactobacillus* species were grouped together irrespective of origin.

### Growth

All *Lactobacillus* strains tested grew better with the sugars than with the sugar alcohols ($p$<0.01 for all), and the growth was usually better with the monosaccharides than with sucrose (Fig. 1). The mean growth from the three sugar substitutes was of the same magnitude within each species, and the lowest mean values were usually seen with xylitol. After 48 h of incubation, the growth (optical density) was somewhat increased (0.04–0.3 units) from the sugars and to a lesser extent from the sugar alcohols where the mean values for *L. rhamnosus*, *L. casei* and the unidentified species were similar to the 24-h values. The overall patterns for the growth with sugars and sugar alcohols were, however, not different.

### Fermentation of glucose, fructose and sucrose

After 24 h of incubation, 74 % of the lactobacilli strains tested could lower the pH to ≤5.5 using glucose and 70 % using sucrose. All strains lowered the pH to <5.5 using fructose. As can be seen in Table 2, the highest pH from glucose, fructose and sucrose was seen for *L. fermentum* strains, while strains of *L. rhamnosus* displayed the lowest pH values. After 48 h of incubation, the mean pH for most strains tested was about 0.2–0.8 units lower compared with the values obtained after 24 h (data not shown).

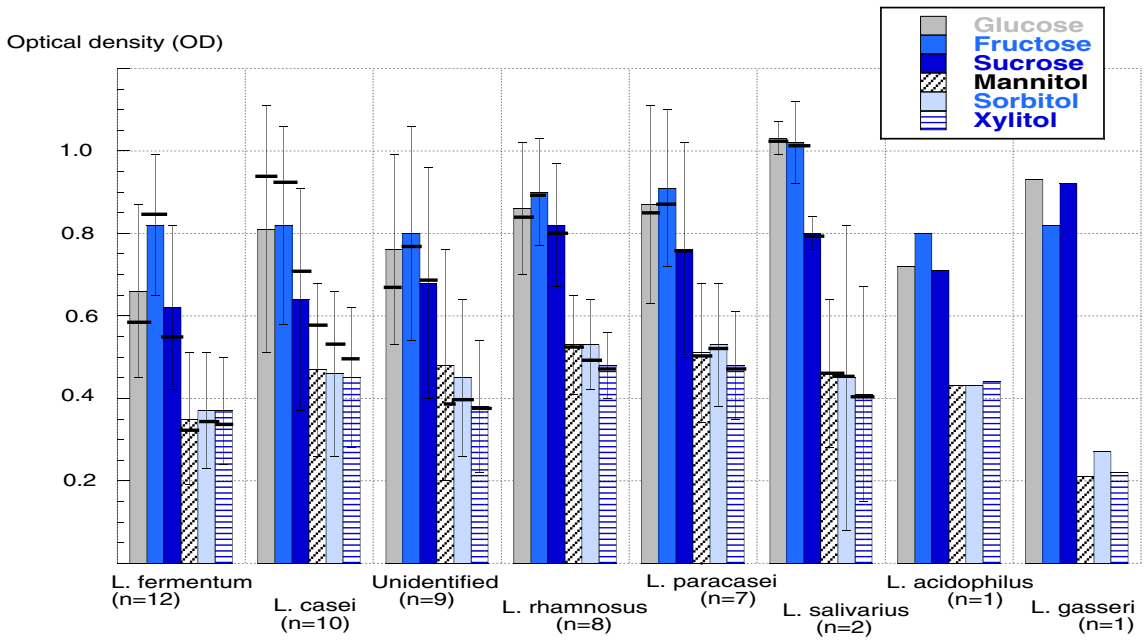


**Fig. 1** Growth (optical density) of the Lactobacillus strains after 24 h of anaerobic incubaction with the sugars and sugar-substitutes. Mean (bars) ± SD and median values (horisontal line)

**Table 2** pH after 24 h of fermentation of glucose, fructose and sucrose for different *Lactobacillus* species

| Species | Glucose | Fructose | Sucrose |
|---|---|---|---|
| *L. fermentum* (*n*=12) | 5.5±0.6[a] (4.6–6.4) (46) | 4.8±0.3[a] (4.3–5.2) (100) | 5.8±0.6[a] (4.6–6.5) (31) |
| *L. casei* (*n*=10) | 4.7±0.9 (4.1–6.5) (80) | 4.2±0.3[b] (3.9–4.7) (100) | 4.9±0.6[b] (4.0–5.9) (80) |
| *L. rhamnosus* (*n*=8) | 4.3±0.2 (4.1–4.7) (100) | 4.2±0.2 (4.0–4.5) (100) | 4.7±0.5 (4.0–5.2) (100) |
| *L. paracasei* (*n*=7) | 4.5±0.7 (4.1–6.1) (86) | 4.2±0.2[c] (4.0–4.6) (100) | 4.8±0.7[c] (4.0–6.1) (86) |
| *L. salivarius* (*n*=2) | 4.4±0.6 (4.0–4.9) (100) | 4.2±0.4 (3.9–4.5) (100) | 4.9±0.0 (4.9–4.9) (100) |
| *L. acidophilus* (*n*=1) | 5.0 | 4.8 | 5.3 |
| *L. gasseri* (*n*=1) | 4.7 | 4.7 | 5.2 |
| Unidentified (*n*=9) | 5.1±0.7 (4.1–6.2) (67) | 4.7±0.5[d, e] (4.1–5.3) (100) | 5.3±0.6 (4.3–6.3) (67) |
| All 50 strains | 4.9±0.8 (4.0–6.5) (75) | 4.4±0.4 (3.9–5.3) (100) | 5.1±0.7 (4.0–6.4) (71) |

Mean ± SD and range are presented as well as proportions of strains giving a $pH \leq 5.5$ (in parentheses)

[a] Higher compared with *L. rhamnosus* ($p<0.001$)

[b] Lower compared with *L. fermentum* ($p<0.01$)

[c] Lower compared with *L. fermentum* ($p<0.001$ for both)

[d] Lower compared with *L. casei* ($p<0.01$)

[e] Lower compared with *L. rhamnosus* ($p<0.01$)

### Fermentation of mannitol, sorbitol and xylitol

As for the sugars, *L. fermentum* displayed the highest pH values and *L. rhamnosus* displayed the lowest at fermentation of sugar alcohols (Table 3). Fifty-two percent of the *Lactobacillus* strains tested lowered the pH to ≤5.5 using mannitol. The ability to ferment mannitol was most common among the *L. rhamnosus* (88 %) and *L. paracasei* (86 %) strains and least common among the *L. fermentum* strains tested (15 %). Forty-nine percent of the *Lactobacillus* strains and especially strains of *L. paracasei* and *L. rhamnosus* lowered the pH to ≤5.5 from sorbitol, while this ability was rarely seen for *L. fermentum.* A pH of ≤5.5 using xylitol was seen for 37 % of all strains; 61 % of the the *L. casei* and *L. paracasei* strains and 38 % of the *L. rhamnosus* strains had this ability. Only one of the *L. fermentum* strains could lower the pH to ≤5.5 using xylitol. For mannitol and sorbitol, the mean pH was 0.1–0.4 pH units lower after 48 h of incubation. The pH after xylitol fermentation had not decreased further after 48 h of incubation.

### Fermentation of sugar substitutes in relation to individuals

*Lactobacillus* strains with the ability to lower the pH to ≤5.5 using all the three sugar substitutes were found in one pSS subject, four RT subjects and two controls, and strains able to lower the pH using mannitol and sorbitol were found in one pSS subject and one RT subject.

## Discussion

In the present study, the in vitro pH-lowering potential of *Lactobacillus* strains, isolated from supragingival plaque from subjects with hyposalivation due to pSS or radiation therapy in the head and neck region (RT) and from healthy

**Table 3** pH after 24 h of fermentation of mannitol, sorbitol and xylitol for different *Lactobacillus* species

| Species | Mannitol | Sorbitol | Xylitol |
|---|---|---|---|
| *L. fermentum* (*n*=12) | 6.4±0.6[a] (5.1–6.9) (15) | 6.5±0.5[a] (5.5–7.0) (8) | 6.5±0.4 (5.7–7.0) (8) |
| *L. casei* (*n*=10) | 5.2±0.7 (4.7–6.8) (80) | 5.6±0.7 (5.0–7.1) (80) | 5.7±0.6 (5.2–7.0) (70) |
| *L. rhamnosus* (*n*=8) | 5.1±0.4 (4.8–6.0) (88) | 5.3±0.3 (5.0–6.1) (88) | 5.6±0.3 (5.2–6.1) (38) |
| *L. paracasei* (*n*=7) | 5.2±0.8 (4.6–6.9) (86) | 5.3±0.7[b] (4.7–6.8) (86) | 5.6±0.6 (4.9–6.9) (57) |
| *L. salivarius* (*n*=2) | 5.5±0.8 (4.9–6.0) (50) | 5.4±0.6 (5.0–5.8) (50) | 5.7±0.7 (5.3–6.1) (50) |
| *L. acidophilus* (*n*=1) | 6.2 | 5.9 | 6.3 |
| *L. gasseri* (*n*=1) | 6.1 | 6.1 | 6.2 |
| Unidentified (*n*=9) | 5.9±0.8 (4.8–6.9) (33) | 6.1±0.7 (5.2–7.0) (22) | 6.2±0.6 (5.4–6.9) (33) |
| All 50 strains | 5.7±0.8 (4.7–6.9) (52) | 5.8±0.7 (4.8–7.1) (50) | 6.0±0.6 (5.1–7.0) (36) |

Mean ± SD and range are presented as well as proportions of strains giving a $pH \leq 5.5$ (in parentheses)

[a] Higher compared with *L. rhamnosus* and *L. casei* ($p<0.01$ for both)

[b] Lower compared with *L. paracasei* ($p<0.01$)

controls, was tested. Initially, it was our intention to compare the strains from hyposalivated subjects with those from controls. However, the strains from the controls were few and they showed similar patterns regarding growth and acid formation as those isolated from hyposalivated subjects. Therefore, strains of the same species were grouped together regardless of origin.

## Methodological considerations

It is difficult to extrapolate the results from the in vitro experiments to the in vivo situation in the mouth. In our experiments, single strains of *Lactobacillus* were tested, while the dental biofilm consists of many different microbial species [12], which compete for space and energy in the in vivo situation. In mature and complex biofilms, the lactic acid produced by for example streptococci, *Actinomyces* and lactobacilli may be consumed by other bacteria like *Veillonella* species [13]. The pH level may therefore not be influenced to the same degree in vivo.

The majority of the *Lactobacillus* strains subjected to fermentations tests were isolated from subjects with hyposalivation and only a few from controls with normal salivary secretion rate. Lactobacilli were rarely detected in the supragingival plaque in the controls [3], and with the method used for random selection, lactobacilli from five controls were isolated and saved. However, in our opinion, the fermentation pattern of lactobacilli in subjects with low salivary secretion rates and high proportions of lactobacilli may be more important to be investigated than lactobacilli in subjects with normal salivary secretion rates and very low numbers of lactobacilli.

The *Lactobacillus* cells were in the exponential growth phase when they were transferred to vials with sugar or sugar alcohols, while the bacteria in the dental plaque are growing at a slow rate with less activity. Also, the time the *Lactobacillus* cells were exposed to the sugar or sugar alcohol, 48 h, differed from the in vivo situation. Studies on unstimulated saliva from healthy children after intake of a xylitol-containing product showed that the xylitol concentration was between 20 and 34 mg/ml 1 min after the intake and remained at a level of >1 mg/ml for at least 16 min [14]. It is likely that the *Lactobacillus* strains in subjects with hyposalivation and a longer oral clearance time have access to a sugar or sugar alcohol at the concentrations of ≥1 mg/ml for even longer periods.

## Sugar alcohols

It is well known that sucrose and other carbohydrates promote the growth of acidogenic and aciduric microorganisms, such as mutans streptococci and lactobacilli [15, 16]. Sugar substitutes are therefore often used to replace sucrose, glucose and fructose. The most commonly used sugar substitutes are mannitol, sorbitol and xylitol, and the two latter are the most frequently used in Sweden. They can be found in oral health care products like toothpaste, fluoride-containing products, mouthwashes, chewing gums and tablets.

It has previously been found that frequent exposure to sorbitol leads to an increased number of sorbitol-fermenting bacteria and a significantly lower pH in dental plaque after frequent sorbitol exposure [17]. The authors state that sorbitol has a cariogenic potential in subjects with impaired salivary secretion rates frequently using sorbitol-containing products [17]. An in vitro study has shown that strains of *L. plantarum* and *L. salivarius* are able to ferment sorbitol to a $pH<5.5$ [9]. In our study, 50 % of the tested strains were able to lower the $pH\leq5.5$ when using sorbitol. This ability was most common among *L. rhamnosus*, *L. casei* and *L. paracasei*. It is therefore possible that these lactobacilli reduce the plaque pH using sorbitol, increasing the risk of caries in hyposalivated subjects.

To our knowledge, the ability of dental plaque bacteria to adapt to xylitol has only been examined in healthy subjects with normal salivary secretion [18]. The authors found that a 2-week period of frequent xylitol use did not lead to adaptation of the dental plaque. However, it should be noticed that this was in healthy subjects who probably had very low numbers of lactobacilli. Badet et al. [9] showed that strains of *L. plantarum* and *L. salivarius* were able to ferment xylitol leading to a $pH<5.5$. They also showed that *Lactobacillus* strains could adapt to xylitol fermentation [10]. In our study, xylitol fermentation was seen for 36 % of the strains and this ability was most common among *L. casei* and *L. paracasei* strains. Eighty-two percent of our *Lactobacillus* strains were isolated from subjects who had suffered from hyposalivation for several years and probably had used fluoride-containing and saliva-stimulating products containing sugar alcohols for a long time. It is possible that these *Lactobacillus* strains had adapted to xylitol fermentation.

Lactobacilli are frequently used as probiotics mostly to treat disturbances in the intestinal microflora [21] but have been suggested in order to improve oral health [22–25]. A probiotic milk containing *L. rhamnosus* GG reduced the number of mutans streptococci and caries lesions in children [22], and *Lactobacillus reuteri* reduced the levels of mutans streptococci in adults [23, 25] and could reduce gingivitis and plaque [24]. The ability to ferment sugars and sugar alcohols has been investigated in 13 probiotic *Lactobacillus* strains [19, 20]. Two strains of *L. plantarum* and *L rhamnosus*, respectively, lowered the pH below 5.2 from sorbitol and one strain, respectively, of *L. reuteri*, *L. rhamnosus* and *Lactobacillus johnsonii* from xylitol. Little is known about the effect of probiotic lactobacilli on the oral microflora of hyposalivated subjects.

In conclusion, we found the ability to produce acids from sugars and sugar substitutes to be generally highest among *L. rhamnosus*, *L. casei* and *L. paracasei* and lowest among the *L. fermentum* strains tested. A large number of *Lactobacillus* strains were able to ferment not only sugars but also the sugar substitutes mannitol, sorbitol and xylitol to pH levels critical for enamel demineralisation. It is therefore possible that frequent use of products containing these sugar alcohols may contribute to the pH-lowering potential of dental plaque and especially in subjects with hyposalivation and high counts of lactobacilli.

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**Conflict of interest** There are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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