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Acid Production by Oral Strains of *Candida albicans* and Lactobacilli

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Key Words
Acid production · Acid tolerance · *Candida* · Cariogenicity · Lactobacilli · pH

Abstract
Both *Candida albicans* and lactobacilli are common colonizers of carious lesions in children and adolescents. The purpose of this study is to compare the velocity of acid production between *C. albicans* and several *Lactobacillus* species at different pH levels and concentrations of glucose. Washed, pure resting-cell suspensions were obtained by culturing a total of 28 oral isolates comprising the species *C. albicans, Lactobacillus rhamnosus, Lactobacillus paracasei paracasei, Lactobacillus paracasei tolerans* and *Lactobacillus delbrueckii lactis*. Acid production from glucose was determined at a constant pH of 7.0, 5.5, 5.0 and 4.0 by repeated titrations with NaOH in an automated pH-stat system. Acid formation rates of yeast and lactobacilli proved to be similar at both neutral and low pH, while in a moderately acidic environment *C. albicans* produced less acid than the lactobacilli. Ion chromatographic analysis of the cell-free medium after titration revealed pyruvate to be the predominant organic acid anion secreted by *C. albicans*. The proportion of organic acids to overall acid production by the yeast was below 10% at neutral conditions, in contrast to 42–66% at pH 4.0. Compared to lactobacilli, yeast required a concentration of glucose that was about 50 times higher to allow acid production at half the maximum speed. Considering the clinical data in the literature about the frequency and proportions of microorganisms present in early childhood caries lesions, the contribution of oral lactobacilli as well as *C. albicans* to overall microbial acid formation appears to be important.

The recognition of acid as the central etiological agent in dental caries initiated a search for the causative microorganism from among the mixed oral microbiota. Lactobacilli along with other microbes, inter alia yeasts, were mainly identified as possible candidates [Tanzer et al., 2001; Wandelt, 1969]. In the early 1960s, mutans streptococci became the main focus of caries research, assumed to be the specific cariogen. However, the occurrence of caries in their absence challenges the specific plaque hypothesis [Kleinberg, 2002]. The more recent ecological plaque hypothesis postulates an ecology-dependent shift in the balance of the resident flora towards microorgan-

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isms which are capable of tolerating and producing acids in larger quantities, thereby causing caries. Mutans streptococci are among the best adapted organisms to the cariogenic environment, but any species with relevant traits can contribute to the disease process [Marsh, 2006]. Lactobacilli are known to be lactic acid producers with exceptional acid tolerance and are widely acknowledged as cariogenic bacteria.

In addition to mutans streptococci and lactobacilli, *Candida albicans* is also strongly associated with dental caries in children, adolescents and young adults [Beighton et al., 2004; Gabris et al., 1999; Moalic et al., 2001]. Although *C. albicans* has been isolated from dentine caries lesions in children with high frequencies ranging from 71 to 97% [de Carvalho et al., 2006; Marchant et al., 2001; Schulz-Weidner et al., 2005; Sziegoleit et al., 2002], it is still not known whether the yeast acts as a caries pathogen or rather plays a role as a commensal microbe. However, *C. albicans* possesses an even broader spectrum of cariogenic properties than lactobacilli: it is capable of adhering to saliva-coated hydroxyapatite [Cannon et al., 1995] and shows strong adherence to collagen [Makihira et al., 2002]. Demonstrating extreme acid tolerance, the yeast proved to lower the pH of glucose-supplemented saliva down to a value of 3.2 by secreting organic acids [Samaranayake et al., 1986]. In a study by Nikawa et al. [2003], *C. albicans* dissolved hydroxyapatite in a liquid culture at a 20-fold higher rate than *Streptococcus mutans,* despite a log 5.9 lower number of yeast cells in the culture.

Another reason for studying yeasts and lactobacilli is their similar distribution in the oral cavity. Both have been isolated more frequently from established dentine caries lesions than from plaque or saliva samples [Nancy and Dorignac, 1992; Sziegoleit et al., 2002]. In the case of lactobacilli, this finding brought about the view that these bacteria are rather responsible for lesion progression than its initiation [Beighton and Brailsford, 1998]. Recent genotyping studies consider lactobacilli to be exogenous and opportunistic colonizers [Caufield et al., 2007].

A crucial criterion for cariogenicity is the amount of acid secreted per unit of time. Therefore, numerous authors have studied pH changes of oral microorganisms during growth. Under such experimental conditions, acid production is influenced by current pH, buffer capacity of the medium, cell density and the phase of microbial growth. Consequently, the results of these studies are difficult to compare and to interpret [Birkhed, 1978]. In an attempt to solve this problem, the velocity of H^+ formation was determined in washed cell suspensions while keeping constant the pH at in vivo relevant levels by addition of alkali [Birkhed, 1978]. This pH-stat method has been applied to study oral streptococci [de Soet et al., 1989, 1991] and *Actinomyces* [Bauer and Kneist, 2005; Kalfas et al., 1990]. Comparable studies on acid production by *C. albicans* have not been published. Kalfas et al. [1990] determined acid formation by various oral bacteria, including three *Lactobacillus* strains at pH 7.0, 6.0 and 5.0. Their results, however, are expressed as proportions of acid produced per nanogram of microbial DNA, which hampers comparison with other studies. Moreover, since the quantification of DNA from Gram-positive oral bacteria is difficult, this method should not be favored. Hence, we employed the pH-stat method to investigate acid production and tolerance of *C. albicans* in comparison with four of the most common oral *Lactobacillus* subspecies.

The parameters of the pH-stat method used in this study were chosen in view of their relevance to pH conditions in the carious lesion. Corresponding clinical data are available for permanent teeth only, and it is debatable to what extent they would also be applicable to deciduous teeth. Dirksen et al. [1962] studied depth-dependent pH within dentinal lesions of permanent teeth. They categorized active cavitated lesions with either small (type I) or large (type II) clinical openings. The pH within the superficial layer of carious dentine ranged from 3.5 to 7.2. The highest values were found on saliva-coated surfaces of type II lesions (mean pH 6.2), while middle layers of type I cavities showed an average pH of 4.2. Based on these data, we decided to measure the velocity of acid production under neutral environmental conditions (pH 7.0) and at three acidic pH levels (5.5, 5.0 and 4.0).

The rate of dental hard tissue demineralization does not only depend on the extent of microbial acid formation, but also on its duration. Fermentable carbohydrates rapidly decrease in concentration after an oral intake due to ingestion and fermentation. Acid formation continues undiminished until the sugar concentration falls below the threshold of saturation that is relevant to the enzymatic processes involved. This means that caries pathogenicity of microorganisms may be influenced by substantial differences in this threshold. For this reason we also investigated the relationship between glucose concentration and acid formation. Additionally, a quantitative analysis of the different organic acids formed by *C. albicans* accompanied this study.

Materials and Methods

Strains and Culture Conditions

A total of 18 isolates of *Lactobacillus* species and 10 strains of *C. albicans* were obtained from samples of saliva or dentine from 26 caries-active children. The lactobacilli were isolated on Rogosa agar and identified as *Lactobacillus rhamnosus* (6 strains), *Lactobacillus paracasei paracasei* (5 strains), *Lactobacillus paracasei tolerans* (5 strains) and *Lactobacillus delbrueckii lactis* (2 strains) according to the fatty acid profiles of their membranes and their biochemical and physiological characteristics [Kneist et al., 1998]. The strains of *C. albicans* were isolated from Sabouraud agar plates and identified employing CHROMagar Candida (Chromagar, Paris, France) as well as by microscopic observation of colonies grown on rice extract agar. Differentiation of *Candida dubliniensis* from *C. albicans* was ascertained by incubation of the cultures at 45°C. One strain which did not grow at 45°C could be identified biochemically by use of Auxacolor (Bio-Rad, München, Germany). Stock cultures were stored at –80°C in Microbank vials (Pro-Lab Diagnostics, Richmond Hill, Ont., Canada) until use.

Previous investigations had shown that acid production by *C. albicans* and *Lactobacillus* species mentioned above was growth phase-dependent and reached its maximum during the late exponential growth of the cultures. Therefore, all strains were grown to the late log phase. After regeneration on Balmelli agar, lactobacilli were cultured under anaerobic conditions (using 95% nitrogen and 5% carbon dioxide as the gas phase) for 16–20 h at 37°C in dextrose broth (1.56% proteose peptone, 0.28% yeast extract, 0.56% NaCl and 1% dextrose). Bacterial cells were harvested by centrifugation and washed 3 times in TS buffer (2 mM triethanolamine hydrochloride in 0.9% saline, pH 7.0). Finally they were resuspended in TS buffer at an optical density of 1.1 at 650 nm and stored on ice until use for a maximum of 5 h.

Candida strains were cultured in dextrose broth at 26°C with shaking (160 rpm) for 18–26 h under aerobic conditions to the late log phase. Once harvested and washed as described above, yeast cells were resuspended in TS buffer to an optical density of 1.6 and stored on ice for a period not exceeding 5 h.

Titration Experiments

Microbial acid production from glucose was monitored at a constant pH of 7.0, 5.5, 5.0 and 4.0 in an automated pH-stat system (Stat titration workstation; Radiometer Analytical SAS, Villeurbanne Cedex, France) based on Birkhed's [1978] method. Five milliliters of the cell suspension was transferred into a thermocontrolled titration vessel and the pH was manually preadjusted with NaOH or HCl. When a temperature of 37°C was reached, 100 μl of prewarmed glucose solution (500 mM in TS buffer) was added. Acid formation was measured for 5 min by recording the volume of NaOH (5 or 10 mM) necessary to maintain the pH at the initial level. The titration volume, pH and temperature of the suspension were automatically recorded every second. Titrations were carried out in triplicate, each time with a fresh aliquot of the same suspension. The dry cell weight was determined for all suspensions and acid production was expressed as the velocity of H^+ produced per minute per milligram of cells.

For the determination of general colony-forming units per cell weight ratios, a representative selection of *Lactobacillus* (n = 10) and *C. albicans* (n = 4) strains were cultured and counted on Rogosa and Sabouraud agar plates, respectively.

The influence of environmental glucose concentration on acid formation was investigated at pH 7.0 and 37°C for one strain each of *L. paracasei tolerans* and *L. rhamnosus* as well as for three *C. albicans* strains. Before titration the yeast cells were stored for 30 min at 37°C to ensure exhaustion of internally stored carbohydrates. Endogenous acid production of lactobacilli could not be observed after the period of temperature equilibration in the titration vessel. Glucose was added to the cell suspensions at different concentrations, immediately followed by titration with NaOH. For the calculation of glucose-dependent velocity of acid production (V_{ap}), the dilution by the titrant was taken into account. Three tests were carried out for each strain at pH 7.0 using separately grown cultures to calculate $K_{0.5}$, which was determined as the concentration of glucose at which half the maximum velocity of acid production ($V_{ap}max/2$) was reached. Differences in pH and temperature during the period of measurement which was used for V_{ap} calculation were not allowed to exceed 0.001 and 0.02 K, respectively, in order to obtain precise results even at low V_{ap}.

Titrations with respect to the analysis of organic acids were performed as outlined above applying *C. albicans* strain DC 24 (now registered as OMZ 1066) suspended in TS buffer lacking NaCl, preadjusted at pH 7.0 or 4.0 with NaOH or HNO_3. Different quantities of glucose were added to give concentrations of 10, 7 and 1.5 mM, respectively, and acid production was recorded until the substrate was totally consumed. All measurements were repeated twice using fresh aliquots of yeast suspension. Following titration, the cells were separated from assay medium by gentle centrifugation and the supernatant was stored at –20°C for subsequent analysis.

Analysis of Acid Anions

Concentrations of organic acids in the assays were determined after appropriate dilution with bidistilled water by the DX 320 ion chromatography (IC) system (Dionex, Sunnyvale, Calif., USA). The IC analyzer was equipped with an EG 40 KOH eluant generator, an IC 20 isocratic pump and conductivity detector. For organic anion separation an IonPac AS15 (2 × 250 mm) analytical column with an IonPac AG15 (2 × 50 mm) guard column (both Dionex) was used. Ion separation was carried out under the following conditions: sample injection volume, 10 μl; eluant flow rate, 0.45 ml min^{-1}; KOH eluant gradients, hold at 5 mM for 3 min, linear from 5 to 30 mM in 7 min, linear from 30 to 45 mM in 10 min, hold at 45 mM for 10 min, return to 5 mM KOH in 2 min and hold at 5 mM for 3 min. The organic acids were quantified with the software Peaknet 5.1 (Dionex) using calibration curves with standard compounds at 20 mg l^{-1}.

Statistical Analysis

Means and standard deviations (SDs) for acid production were calculated to describe the data. The variance of V_{ap} increased with higher pH values. Therefore, statistical analyses were done using log-transformed data, for which homoscedasticity was not violated (Levene's test: p = 0.349). The influences of the species and the environmental pH on acid production were analyzed using a cell means model including main and interaction effects. Resulting estimations for means and their 95% confidence intervals were transformed back to the original scale by antilogarithm; p values less than 0.05 were considered statistically significant. All these analyses were performed with SPSS 14.0 (SPSS Inc., Chicago, Ill., USA).

Results

The V_{ap} values for each species at different environmental pH values are presented in figure 1a. All strains exhibited the highest V_{ap} at pH 7.0. The reduction of environmental pH significantly decreased the rate of V_{ap} ($p < 0.001$).

L. delbrueckii showed the highest V_{ap}, significantly different from *L. rhamnosus* at each pH level ($p < 0.05$). *L. paracasei paracasei* produced less acid than *L. delbrueckii* (statistically significant at pH 4.0, 5.0 and 5.5), but showed faster acid formation than *L. rhamnosus* (not significant at pH 5.5). *C. albicans* exhibited a higher V_{ap} than both *L. rhamnosus* and *L. paracasei paracasei* at pH 7.0, whereas in acidic environment less acid was formed by the yeast than by the *Lactobacillus* species (for statistical significance, see fig. 1a).

Acidogenesis of all *Lactobacillus* species dropped almost linearly with decreasing pH. In contrast, the V_{ap} of *C. albicans* decreased faster ($p < 0.001$), but regressively (fig. 1b). Therefore, the acid formation rates of yeast and lactobacilli proved to be different ($p < 0.05$) in a moderately acidic environment (pH 5.5 and 5.0), but were similar at both neutral (7.0) and low pH (4.0).

Strains with average species-related acid production were selected to study the effect of changes in environmental glucose concentration. Limiting the substrate influenced the acid formation of lactobacilli and *C. albicans* to a widely different extent (fig. 2). *L. paracasei tolerans* and *L. rhamnosus* produced acid with a $K_{0.5}$ of 11.3 ± (SD) 0.4 μmol l^{-1} and 14.1 ± 1.3 μmol l^{-1}, respectively. In contrast, the three *C. albicans* strains exhibited $K_{0.5}$ of 480 ± 100, 630 ± 97 and 840 ± 20 μmol l^{-1}, respectively, which were on average about 50 times higher than the $K_{0.5}$ of the lactobacilli.

As determined by ion chromatography, pyruvic acid was the most abundantly excreted organic acid at every condition, except at low initial glucose concentration at pH 7.0 for *Candida*, where acetic acid was dominant (fig. 3). Pyruvate and α-ketoglutarate were equally excreted at pH 7.0 and 4.0, while malate was exclusively found in the acidic medium and acetate was present to a greater extent at neutral conditions. Additionally, traces of formate, lactate and citrate were identified. No other organic acids were observed above the threshold of detection (0.5 mg l^{-1}). Low extracellular concentration of glucose changed the pattern of acid extrusion at both pH.

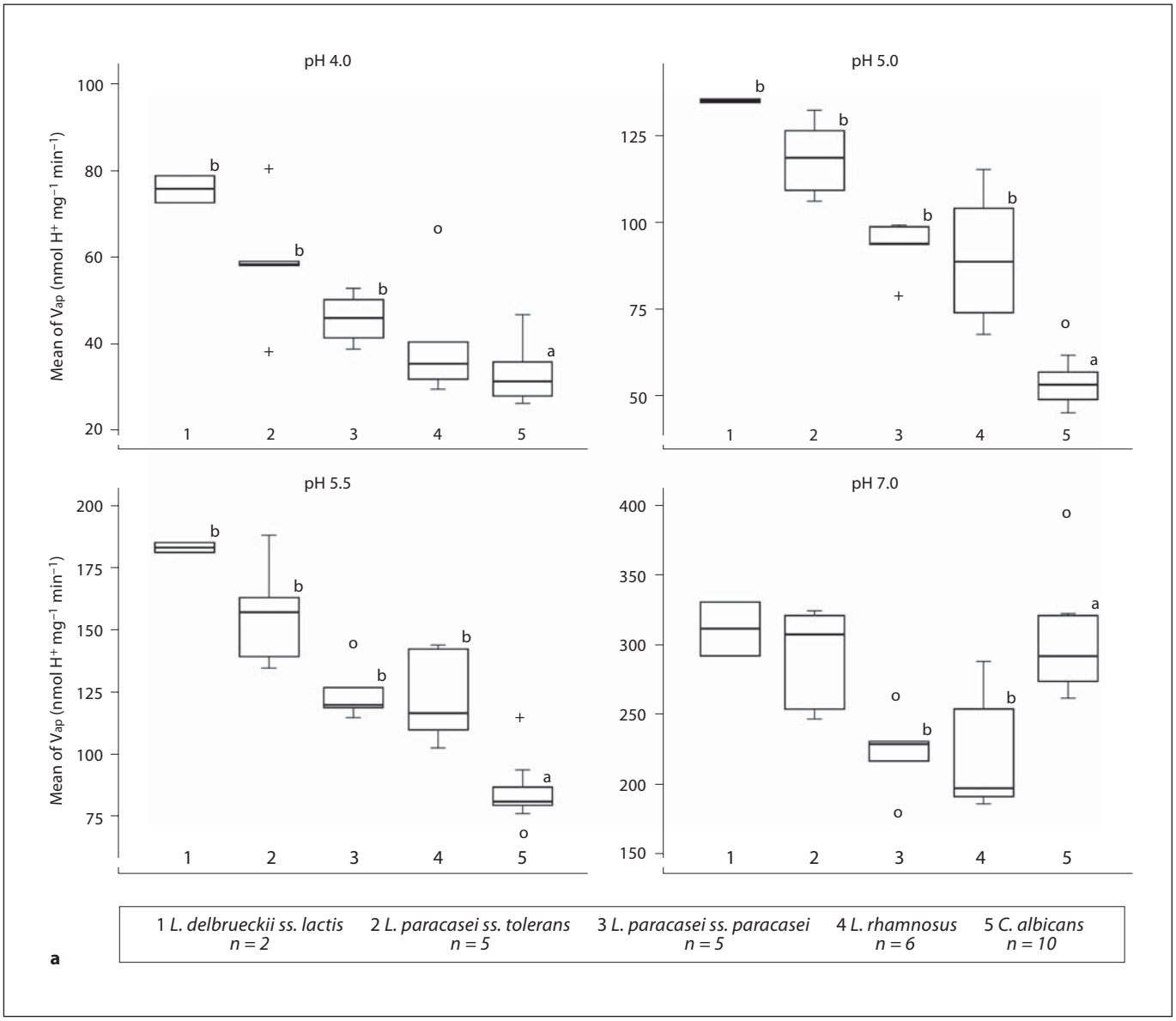
The quantities of H^+ which became effective in the assay medium due to the accumulation of different organic acids are indicated in table 1. The proportion of cations derived from dissociated organic acids to the total titratable acid was only 7–8% in neutral conditions, while at pH 4.0 more than 60% of the total acid originated from organic acids in the case of high or medium initial concentration of glucose. This difference did not accrue from the organic acids which were excreted by the yeast almost independently of its environmental pH, but from a considerable decrease in total acid formation measured by titration when the preset pH of the medium was lowered from 7.0 to 4.0 (table 1).

Discussion

While lactobacilli are considered cariogenic because of their acidogenicity and high prevalence in cavitated carious lesions, caries causation by *C. albicans* in spite of the same premise has been a matter of speculation. The present study provides quantitative data of in vitro acid production and tolerance of *C. albicans* and lactobacilli employing the pH-stat method and considers effects of substrate concentration as well as acidic metabolites formed by the yeast.

At neutral pH, acid formation of *C. albicans* and lactobacilli proved to be moderate compared to *S. mutans*, for which V_{ap} levels of 0.27–0.9 μmol min^{-1} mg^{-1} were reported in previous studies of similar design [de Soet et al., 1989, 1991; Kneist et al., 2005]. However, in an environment with a pH below 5.5, which is relevant for caries formation, acidification by *S. mutans* decreased considerably and ceased around pH 4.2 [de Soet et al., 1991], whereas both *C. albicans* and lactobacilli were still secreting acid in significant quantities at pH 4.0. Oral streptococci of the mutans group showed a decrease in V_{ap} between 55% *(Streptococcus rattus)* and 47% *(Streptococcus sobrinus)* when the environmental pH was lowered from 5.5 to 5.0 [de Soet et al., 1989]. With the same conditions in the present study, acid production of *C. albicans* was reduced by only 36%, and lactobacilli showed an even smaller decline ranging between 30 and 24%. This exceedingly high acid tolerance may favor lactobacilli and *C. albicans* in microbial competition, particularly when they are resident in deep dentine caries lesions with small openings, where Dirksen et al. [1963] measured pH values below 4.5 in 71% of the lesions within subsurface layers and in even 90% at the cavity base.

The obvious nonlinear correlation between pH and V_{ap} of *C. albicans* highlights the complexity of acid formation by the yeast. In contrast to lactobacilli in which acidogenicity is mainly based on the secretion of lactic



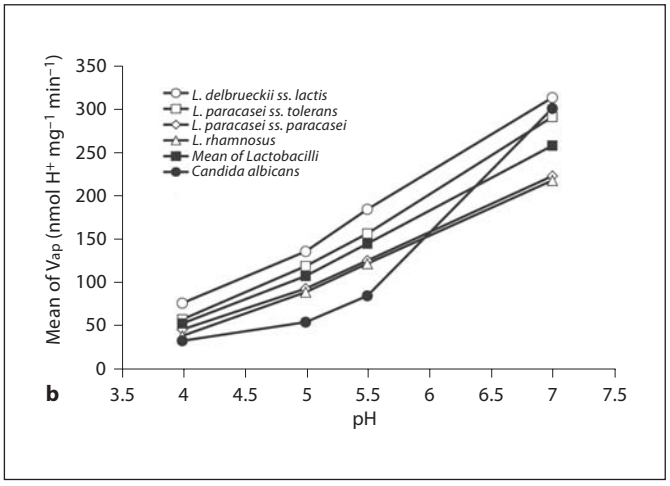


Fig. 1. Acid production by *Lactobacillus* and *C. albicans* strains related to dry cell weight. **a** Median, 25th and 75th percentile, minimum and maximum values are shown for each of four environmental pH levels. Outliers are marked by circles and extreme values by plus signs. Significant differences between *C. albicans* and *Lactobacillus* species ($p < 0.05$) are indicated for each pH level by different characters (a, b). Note the dissimilar y-axes. **b** Dependence of acid production on pH by *C. albicans* compared to four different *Lactobacillus* species and the mean of lactobacilli.

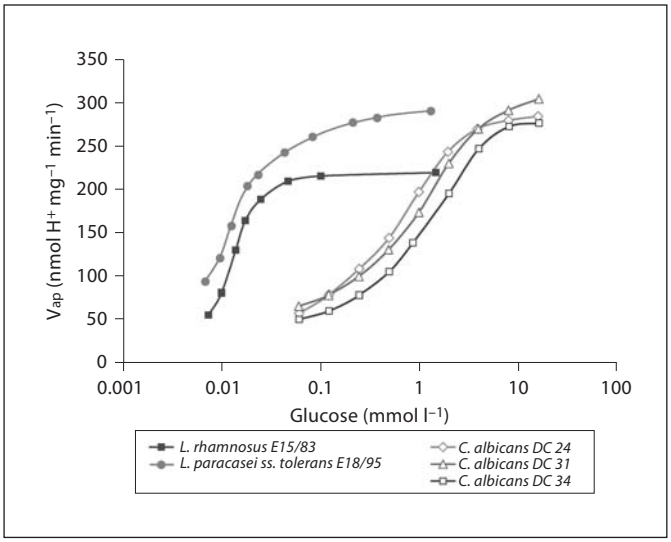


Fig. 2. Acid formation by *L. paracasei tolerans* E 18/95, *L. rhamnosus* E 15/83 and *C. albicans* strains DC 24, DC 31 and DC 34 at pH 7.0 and different environmental concentrations of glucose.

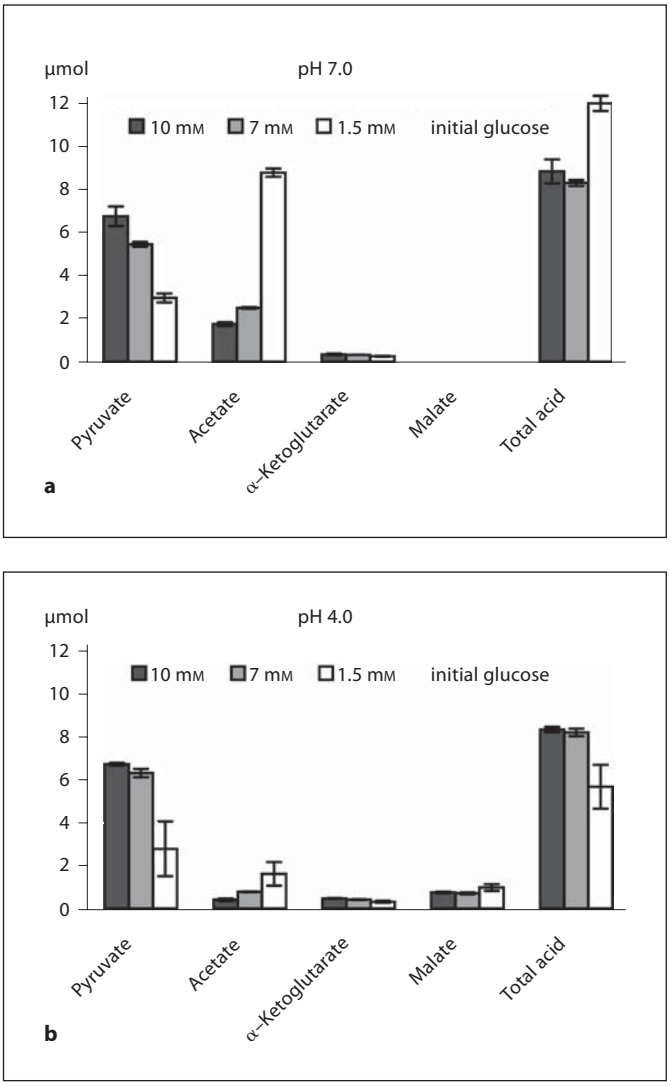


Fig. 3. Organic acid anions released into the assay medium by *C. albicans* strain DC 24 at pH 7.0 (**a**) and 4.0 (**b**). Glucose was added with initial concentrations of 10, 7 or 1.5 mM and allowed to be totally metabolized by the yeast. The quantities of acids were calculated assuming an equal consumption of 100 µmol of glucose. Means and SDs of three tests are shown.

acid, there are at least three different processes that participate in the acidification of a surrounding environment by yeast cells:

Firstly, ion chromatographic analysis revealed the extrusion of several organic acids by *C. albicans*. Because of its low pK_a of 2.39, the predominant pyruvic acid (fig. 3) is even more potent to reduce the pH of an already intensely acidified environment than lactic acid (pK_a = 3.86). Pyruvate and acetate were also detected by Samaranayake et al. [1986] to be the major acidic components in glucose-supplemented saliva. Collings et al. [1991] found pyruvate most abundantly among other organic acids during the late log phase of growth in a synthetic medium, whereas later in the stationary phase acetate accumulated to high amounts while pyruvate decreased, apparently due to reutilization by the yeast. Regarding the effect of organic acids formed by a microbial community in vivo, reutilization processes have to be considered as well as the inhibition of acid production by accumulated acid anions at low pH as shown for oral streptococci [Dashper and Reynolds, 2000]. Investigations with respect to lactobacilli and *C. albicans* are in progress.

At physiological pH, organic acids apparently play a minor part measured against overall acid production (table 1). In carious dentin, however, where acidic conditions persist over extended periods [Hojo et al., 1994], the pH-independent excretion of strong organic acids becomes the main factor in acidogenicity, as shown in table 1 for pH 4.0. This high acidic potential of *C. albicans* at low pH is also expressed by the low decrease in V_{ap} between pH 5.0 and 4.0 when compared with the lactobacilli (fig. 1b).

Acetate has been observed to be the predominant acid anion in resting plaque fluid [Margolis et al., 1988] and in arrested caries lesions [Hojo et al., 1994]. Under analogous experimental conditions in the present study re-

Table 1. Effective release of H^+ ions into the assay medium by *C. albicans* strain DC 24 (OMZ 1066) uniformly calculated corresponding to the consumption of 100 μmol of glucose at pH 7.0 and 4.0

pH	Initial concentration of glucose, mM	H^+ from pyruvate, μmol	H^+ from acetate, μmol	H^+ from α-ketoglutarate, μmol	H^+ from malate, μmol	Total H^+ from organic acids, μmol	Total H^+ measured by titration, μmol	H^+ ratio of organic to titrated acids, %
7.0	10	6.76 (0.46)	1.74 (0.08)	0.68 (0.07)	0	9.18 (0.59)	114.7 (1.64)	8.0
7.0	7	5.48 (0.11)	2.49 (0.04)	0.64 (0.01)	0	8.61 (0.14)	121.7 (2.43)	7.1
7.0	1.5	2.98 (0.21)	8.72 (0.19)	0.51 (0.05)	0	10.71 (0.36)	147.3 (5.39)	7.3
4.0	10	6.47 (0.07)	0.05 (0.01)	0.52 (0.02)	0.74 (0.03)	7.78 (0.06)	12.6 (0.21)	61.6
4.0	7	6.08 (0.18)	0.10 (0.00)	0.46 (0.02)	0.71 (0.05)	7.33 (0.16)	11.2 (0.29)	65.7
4.0	1.5	2.68 (1.23)	0.20 (0.07)	0.34 (0.05)	0.97 (0.16)	4.19 (1.33)	9.9 (1.64)	42.4

Glucose was added with initial concentrations of 10, 7 and 1.5 mM and allowed to be totally metabolized by the yeast. Cation quantities were calculated both for organic acids from their anionic equivalents determined by ion chromatography considering the degree of dissociation at the respective pH and for total acid from NaOH equivalents added during pH-stat titration. SD is shown in parentheses.

garding high pH and low glucose, *C. albicans* also produced mainly acetate.

Secondly, the plasma membrane of yeasts is abundantly equipped with an H^+-ATPase which actively pumps out protons from the cell to generate an electrochemical gradient which is used in the co-transport of nutrients [Bowman and Bowman, 1986]. The extrusion of protons is induced by glucose [Serrano, 1983] and very likely makes a major contribution to the net acidification. This is confirmed by studies on H^+-ATPase inhibitors which caused a considerable decrease in the acidification of the external medium by whole yeast cells [Kotyk et al., 1999; Manavathu et al., 1999].

If the uptake of glucose into the cell controls the speed of H^+ formation in a stoichiometric ratio as shown for *Candida utilis* [van den Broek et al., 1997], differences in V_{ap} should be reflected by the glucose consumption rates of the organisms. Basson [2000] found the glucose consumption rate of *C. albicans* to be almost half the rate of *Lactobacillus casei*, which indeed corresponds to the about two times lower V_{ap} of the yeast compared to the lactobacilli at acidic pH in the present study (fig. 1b).

A third source of acidification is based on the excretion of carbon dioxide resulting from oxidative or fermentative glucose metabolism. In an aqueous medium, CO_2 is partially dissolved forming carbonic acid (pK_{a1} = 6.36), which in neutral and alkaline conditions dissociates to produce bicarbonate and hydrogen ions. Presumably this process is responsible for the massive rise in V_{ap} at pH 7.0 measured in this study (fig. 1b). The in vivo acidification by carbonic acid is difficult to predict; admittedly, the real acid formation could be even higher than measured by the pH-stat method, since the pH of the medium, artificially held constant at 7.0 by the addition of OH^-, shifts the dissociation equilibrium between H_2CO_3 and CO_2 towards CO_2, causing its enhanced release in gaseous form.

Corresponding to the differences in the mode of acid production, *C. albicans* and lactobacilli were differently influenced in their V_{ap} by the concentration of glucose. Plaque bacteria like mutans streptococci and lactobacilli use the phosphoenolpyruvate-phosphotransferase system for the uptake of mono- and disaccharides [Ajdic and Pham, 2007; Barrangou et al., 2006]. Therefore, it is not surprising that the $K_{0.5}$ values obtained for *L. paracasei tolerans* (11 μmol l^{-1}) and *L. rhamnosus* (14 μmol l^{-1}) are in accordance with comparable data for *S. mutans* (20 μmol l^{-1} [Stoesser, 1984]) and dental plaque (12 μmol l^{-1} [Stoesser, 1984], 20 μmol l^{-1} [Roberts and Hayes, 1980]). In contrast, sugar uptake of *C. albicans* is dependent on the H^+-ATPase system which is apparently activated at a 50-fold higher concentration of glucose. This implies that undiminished acid production by *C. albicans* is restricted to those periods of time when sugars are available in at least millimolar concentrations within the dental plaque.

The time-dependent availability of sugars is determined by the oral clearance, which denotes the elimination of sugars from the oral cavity. On the basis of a mathematical model of salivary sugar clearance by Dawes [1983] as well as clinical data from Singer and Kleinberg [1983] on the retention times of glucose in dental plaque,

it can be estimated that the $K_{0.5}$ concentration determined for *C. albicans* (480–840 μmol l^{-1}) is reached about twice as fast as the one measured for lactobacilli (11–14 μmol l^{-1}). Thus, *C. albicans* very likely contributes exclusively to the first half of an individual sugar-induced pH fall. Despite the fact that the sugar clearance from open cavities is not properly described by the model mentioned above and may be distinctly reduced in case of adhesive food, the difference between the $K_{0.5}$ values of yeasts and cariogenic bacteria certainly has a sizeable influence on the duration of acid production by the respective microorganisms following an oral sugar intake.

The availability of utilizable carbohydrates plays an important role in microbial competition. Hence, the large requirement for glucose may be a limiting factor for the establishment of yeasts within the microbial community of a carious lesion. Knight and Fletcher [1971] observed a distinctly restricted growth of *C. albicans* in natural human saliva containing viable bacteria, in contrast to rapid growth when the saliva was previously sterilized. Addition of glucose, however, stimulated the yeast to grow in the natural saliva also.

Basson [2000] investigated the competition for glucose as a growth-limiting substrate in a chemostat. Under glucose-excess conditions, *C. albicans* and *L. casei* became established in a mixed community of oral bacteria. However, in a glucose-limited chemostat the yeast proved unable to compete for a niche while *L. casei* successfully maintained a steady state in the mixed culture.

Several clinical and animal investigations have shown that oral colonization by *C. albicans* can be promoted by a sugar-rich diet [Samaranayake, 1986]. In a study of diabetic children and adolescents, the presence of yeasts in saliva was highly associated with poorly controlled diabetes, while lactobacilli counts were not dependent on blood glucose levels [Karjalainen et al., 1997].

The extent to which *C. albicans* may contribute to cariogenesis has to be considered in the context of its proportion in the overall microflora. Marchant et al. [2001] studied the microbiology of 52 caries lesions in deciduous teeth and found *C. albicans*, lactobacilli and mutans streptococci to be similarly frequent; but the portion of *C. albicans* was merely 0.2%, while lactobacilli represented 1.3% and mutans streptococci 6.0% of the total cultivable flora. With regard to the distinctly different cell dimensions, however, microbial proportions should be considered on the basis of their biomass. According to our own investigations, 1 CFU of *C. albicans*, *Lactobacillus* spp. and *S. mutans* proved to have a dry weight of 17.4, 3.5 and 0.41 pg, respectively. The calculated biomass of these microorganisms using the data of the clinical study mentioned above results in a ratio of 1:1.3:0.7, presenting yeasts to be even more extensive in nursing caries lesions than mutans streptococci.

It can be assumed from these findings that due to acid production *C. albicans* makes a significant contribution to caries pathogenesis in caries-active children. Additional capabilities of the yeast such as the formation of hyphae and the secretion of dentine-degrading enzymes [Hagihara et al., 1988; Klinke et al., 2007] must be taken into account for an appraisal of caries pathogenicity.

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