Inhibitory effect of sodium fluoride and chlorhexidine on the growth of oral lactobacilli

María del Carmen Ahumada Ostengo, Birgitt Wiese, and María Elena Nader-Macias

Abstract: The accumulation of microorganisms in dental plaque is related to the etiology of caries and periodontal disease, with a high prevalence worldwide. The prophylactic measures include the use of chemical agents as NaF and chlorhexidine. Lactic acid bacteria are members of the normal microbiota of the oral cavity being discussed with regard to their beneficial or detrimental effect in this environment. The present study was performed to determine the growth of some species of *Lactobacillus* at different concentrations of NaF and chlorhexidine. The strains were isolated from both caries-free and caries patients. Their growth parameters were evaluated by the application of the Gompertz model to the experimental data of optical density as a measurement of growth. The degree of inhibition of the growth of all of the lactobacilli studied was different, depending on each particular strain. NaF at 1 mmol·L^{-1} inhibited between 5% and 46%, at 5 mmol·L^{-1} between 13% and 65%, and at 20 mmol·L^{-1} between 57% and 84%. CHX at higher concentrations (197 and 98 mmol·L^{-1}) showed a complete inhibition of some of the strains. The significance of the results was evaluated by the application of a multivariate analysis and also compared with the inhibition of pathogenic *Streptococcus mutans* and with lactobacilli strains from collection cultures.

Key words: oral lactobacilli, sodium fluoride, chlorhexidine, growth inhibition, growth parameters, Gompertz model.

Résumé : L'accumulation de micro-organismes dans la plaque dentaire est reliée à l'apparition des caries et des maladies parodontales, avec une prévalence élevée partout dans le monde. Les mesures préventives englobent l'utilisation d'agents chimiques comme le fluorure de sodium (NaF) et la chlorhexidine (CHX). Les bactéries lactiques font partie de la microflore normale de la cavité orale et ont des effets bénéfiques ou néfastes sur cet environnement. La présente étude a été accomplie afin de déterminer la croissance de certaines espèces de lactobacilles à différentes concentrations de NaF et de CHX. Les souches ont été isolées de patients avec ou sans caries. Leurs paramètres de croissance ont été évalués en appliquant le modèle de Gompertz aux données expérimentales de densité optique (D.O.) afin d'obtenir une mesure de la croissance. Le niveau d'inhibition de la croissance des lactobacilles étudiées différait selon la souche. 1 mmol·L^{-1} de NaF a inhibé entre 5 % et 46 %, entre 13 % et 65 % pour 5 mmol·L^{-1} et entre 57 % et 84 % pour 20 mmol·L^{-1}. Des concentrations élevées de CHX (197 et 98 mmol·L^{-1}) ont complètement inhibé certaines souches. La signification des résultats a été évaluée par une analyse multivariable de même que par la comparaison avec l'inhibition de *Streptococcus mutans* pathogènes et de souches de lactobacilles issues de cultures de collections.

Mots clés : lactobacilles orales, fluorure de sodium, chlorhexidine, inhibition de la croissance, paramètres de croissance, modèle de Gompertz.

[Traduit par la Rédaction]

Introduction

Dental plaque is found on healthy enamel but is also implicated in the etiology of 2 of the most prevalent diseases affecting industrial societies: caries and periodontal disease. Prevention of these pathological situations involves the constant mechanical removal of plaque from the tooth surface, although the difficulty in maintaining a striking mechanical oral regime suggests that effective chemical adjunctive measures would significantly contribute to overall oral health. There is therefore considerable interest in the use of antiplaque and or antimicrobials agents in the prevention and treatment of these diseases (Pratten et al. 1998).

Fluoride is frequently employed as an antiplaque agent. It has been studied thoroughly in past years. The anticaries properties of fluoride are thought to include effects on both calcium phosphate chemistry and bacterial metabolism (Wahab et al. 1993). The most important effect of fluoride on bacterial physiology is the inhibition of glycolysis, mainly at low pH. First, fluoride inhibits enolase, which reduces both glycolytic rate and transport of sugar into bacteria (Hamilton 1977). Furthermore, it acidifies the interior of

Received 16 July 2004. Revision received 16 July 2004. Accepted 22 November 2004. Published on the NRC Research Press Web site at http://cjm.nrc.ca on 15 April 2005.

M.C. Ahumada Ostengo and M.E. Nader-Macias.[1] CERELA-CONICET, Chacabuco 145, 4000, Tucumán, Argentina.
B. Wiese. Institute of Biometry, Medical University of Gottinger, Hannover, Germany.

[1]Corresponding author (e-mail: fnader@cerela.org.ar).

 doi: 10.1139/W04-128

the cells and inactivates some enzymatic metabolic processes and it inhibits the synthesis of intracellular reserves such as glucogen (Wahab et al. 1993).

Chlorhexidine (CHX) has been used extensively in the control of plaque (Löe et al. 1976; Loesche 1976; Emilson 1977, 1981; Schaeken et al. 1984) and the colonization of the mouth by *Streptococcus mutans* (Emilson 1981; Schaeken et al. 1984). The daily application of mouthwash with CHX reduces dental plaque, gingivitis, and cavities in the oral cavity (Emilson 1994; Bowden 1996; Pratten et al. 1998). CHX is the agent that is used more frequently against *S. mutans*; it is presented commercially in the form of mouthwash, gels, and varnishes. Natural susceptibility to CHX varies, being more potent on Gram-positive than on Gram-negative microorganisms (Hennessey 1973; Emilson 1977; Stanley et al. 1989).

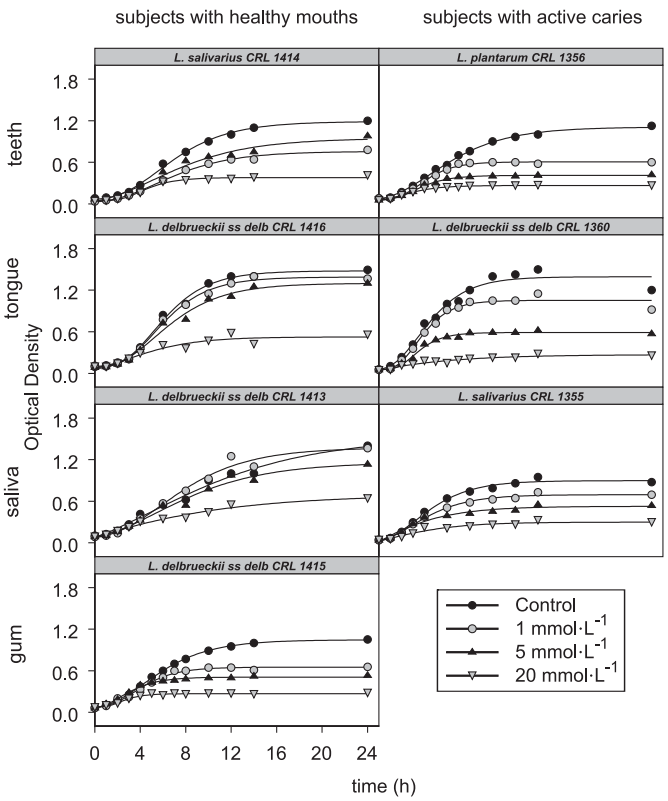
In previous papers, the isolation, phenotypic identification, and probiotic properties of lactobacilli from different areas of the mouth have been reported (Ahumada et al. 1999, 2001; Colloca et al. 2000). The differences between the strains isolated from caries-free and caries patients have also been reported (Ahumada et al. 2003) together with their characteristics to be used as probiotic strains. The selected strains were phenotypically identified and included in the Centro de Referencia para Lactobacilos (CERELA) Culture Collection strains. The main interest of our group, from an ecological point of view, was to study the behavior of lactobacilli isolated from 2 different groups of patients or from diverse areas of the oral cavity growing at different concentrations of NaF and CHX to further evaluate the possibility of their inclusion in probiotic products to be applied to the oral cavity. The evaluation of their growth parameters was performed by the application of the Gompertz model, applied to changes in optical density (OD) as a measurement of growth. Also, the effect of these substances on the growth of *S. mutans* ATCC 25175 and on culture collection lactobacilli strains was determined to subsequently make some predictions on the behavior of these microorganisms in the oral cavity.

Materials and methods

Microorganisms

Lactobacilli were isolated from the oral cavity of caries-free and caries patients from Tucumán, Argentine, published previously. Sample collection was performed from 4 sites of the oral cavity: teeth, tongue, gum, and saliva (Ahumada et al. 1999; Colloca et al. 2000). The strains used were *Lactobacillus delbrueckii* ssp. *delbrueckii* (Centro de Referencia para Lactobacilos (CRL) 1360, CRL 1416, CRL 1413, and CRL 1415), *Lactobacillus salivarius* (CRL 1355 and CRL 1414), and *Lactobacillus plantarum* (CRL 1356 and CRL 1363) deposited in the CERELA (Centro de Referencia para Lactobacilos) Strain Culture Collection. American Type Culture Collection (ATCC) strains were also used for comparison: *Lactobacillus salivarius* ssp. *salivarius* ATCC 11741, *L. delbrueckii* ssp. *delbrueckii* ATCC 9649, and *L. plantarum* ATCC 14917. *Streptococcus mutans* ATCC 25175 was also used.

Fig. 1. Effect of different concentrations of NaF on lactobacilli of subjects caries free and subjects with caries.



Growth conditions

Before the experiments, each strain stored in milk – yeast extract at –70 °C, was propagated in LAPTg (Raibaud et al. 1973) (1.5% peptone, 1% tryptone, 1% glucose, 1% yeast extract, and 0.1% Tween 80) at 37 °C for 24 h and subcultured twice at 37 °C for 12 h. The last preculture was washed with saline solution (0.85% NaCl) and resuspended in the same solution to give an OD of 1.2–1.4 at 540 nm (10^8–10^9 colony-forming units (CFU)/mL. This suspension was used as the inoculums for the growth experiments. The lactobacilli were inoculated at 2% *v/v*.

Evaluation of the effect of NaF and CHX digluconate

Bacterial suspensions were added to broth (5 mL) containing different concentrations of NaF, 1, 5, and 20 mmol·L^{-1}, added before autoclaving. The same bacterial suspensions were added to broth (5 mL) with different concentrations of CHX digluconate, 197.8, 98.8, 24.7, and 2.47 mmol·L^{-1}, added after autoclaving in sterile conditions. The initial pH of the LAPTg broths was adjusted to 6.5. Samples were taken at specific time intervals to determine the OD at 540 nm in glass tubes by using a spectrophotometer Spectronic 20™ (Bausch & Lomb; Thermo Electron Corp., Madison, Wisconsin).

The experiments were performed each day with 1 microorganism, and the growth curves were repeated 2 or 3 times on different days.

Table 1. Estimation of the growth parameters by the application of the Gompertz model to the experimental results of the growth of oral lactobacilli and the inhibitory effect of NaF at different concentrations.

Strain	Concentration ($mmol \cdot L^{-1}$)	N_0	$A \pm SE$	$\mu \pm SE$	$\lambda \pm SE$
L. delbrueckii ssp. *delbrueckii* CRL 1360	0	0.04	1.35±0.12	0.21±0.02	1.20±0.50
	1	0.053	0.99±0.08*	0.22±0.09	1.61±0.49
	5	0.05	0.53±0.06*	0.14±0.03*	1.62±0.59
	20	0.04	0.21±0.02*	0.02±0.01*	0.01±1.01
L. salivarius CRL 1355	0	0.004	0.89±0.04	0.12±0.01	0.69±0.27
	1	0.001	0.69±0.02*	0.09±0.01	0.43±0.43
	5	0.001	0.49±0.39*	0.08±0.02	0.02±0.84
	20	0.02	0.27±0.03*	0.04±0.05*	0.001±1.13
L. plantarum CRL 1356	0	0.02	1.08±0.04	0.10±0.004	0.79±0.79
	1	0.08	0.79±0.02*	0.11±0.03	1.90±0.46
	5	0.05	0.35±0.04*	0.07±0.01*	1.33±0.65
	20	0.03	0.23±0.015*	0.04±0.01*	0.12±0.27
L. delbruekii ssp. *delbruekii* CRL 1416	0	0.10	1.37±0.12	0.19±0.02	2.55±0.57
	1	0.08	1.30±0.06	0.18±0.01	2.46±0.38
	5	0.05	1.24±0.06*	0.14±0.01*	1.97±0.51
	20	0.04	0.47±0.18*	0.05±0.02*	0.001±1.09*
L. delbrueckii ssp. *delbrueckii* CRL 1415	0	0.04	1.00±0.03	0.11±0.002	1.10±0.19
	1	0.07	0.57±0.05*	0.10±0.02	1.39±0.83
	5	0.04	0.45±0.04*	0.09±0.01*	0.45±0.32*
	20	0.07	0.19±0.03*	0.06±0.13*	1.08±0.81*
L. salivarius CRL 1414	0	0.05	1.12±0.06	0.12±0.01	2.14±0.51
	1	0.02	0.73±0.06*	0.07±0.01*	1.68±0.46
	5	0.001	0.94±0.13*	0.07±0.02*	0.67±0.96
	20	0.04	0.33±0.02*	0.07±0.02*	2.18±0.64
L. delbrueckii ssp. *delbrueckii* CRL 1413	0	0.006	0.08±0.01	0.08±0.01	0.001±0.68
	1	0.053	1.32±0.4	0.11±0.02*	1.66±0.93
	5	0.007	0.93±1.06*	0.082±0.02	0.24±1.52
	20	0.07	0.57±0.51*	0.04±0.01*	0.001±1.00

Note: N_0: initial data; A, incremented OD between D_0 and OD_{max}; μ, maximum growth rate (h^{-1}); λ, duration time of lag phase.
*Effect statistically significant.

Estimation of growth parameters

The bacterial growth parameters were estimated applying the four-parameter modified Gompertz model represented by the following expression:

$$D_t = D_0 + A \exp\{-\exp[(\mu e/A)(\lambda - t) + 1]\}$$

where D_t is the OD at time t (time of growth in hours), D_0 is the OD at $t = 0$, A is the increase in OD between D_0 and OD_{max}, μ is the maximum specific growth rate (per hour), λ is the lag phase (hours), and "e" is the base of the neperian logarithm (Zwietering et al. 1990).

To estimate the parameters, a constrained nonlinear regression was performed. This method uses a sequential quadratic programming algorithm. For the standard errors and confidence intervals of the parameters, the method of bootstrapping was applied using repeated samples from the original data set. For each bacterial growth curve, 100 bootstrap samples were taken.

More detailed mathematical expressions supporting the application of the model have been published previously (Juarez Tomás et al. 2002).

Statistical evaluation

The differences in the growth parameters obtained when determining the effect of the different concentrations of each substance and the control were calculated including the differences as parameters directly in the model equation. This step needs to be performed to estimate the confidence intervals for the difference between parameters and to make assumptions about the statistical significance of the inhibitions.

To evaluate the multivariate effects of the different conditions, e.g., concentration, origin, and health status, on the growth parameters, the nonlinear mixed-effects model as proposed by Lindstrom and Bates (1990) was applied using restricted maximum likelihood.

For the analyses and graphical presentations, the statistical programs SPSS 12 and S-plus 6.0 were used.

Results

Effect of NaF on the growth of oral lactobacilli

The results obtained when applying the nonlinear regression method to the experimental data of NaF on growth are shown in Fig. 1. In this figure, the effects of all of the con-

Table 2. Inhibition percentages of NaF and CHX on the growth of ATCC and CRL strains.

	NaF (mmol·L^{-1})			CHX (mmol·L^{-1})			
Strain	20	5	1	197.8	98.8	24.7	2.47
L. plantarum ATCC 14917	75	60	50	95	93	12	6
L. plantarum CRL 1356	78	67	26	99	99	0	0
L. plantarum CRL 1363	nd	nd	nd	99	99	0	0
L. salivarius ssp. *salivarius* ATCC 11741	80	65	41	94	36	5	5
L. salivarius CRL 1355	69	44	22	99	99	33	9.4
L. salivarius CRL 1414	70	16	34	98	98	11	8.9
L. delbrueckii ssp. *delbrueckii* ATCC 9649	92	74	64	96	95	10	10
L. delbrueckii ssp. *delbrueckii* CRL 1360	84	60	26	99	81	27	0
L. delbrueckii ssp. *delbrueckii* CRL 1416	65	9	5	79	53	31	3
L. delbrueckii ssp. *delbrueckii* CRL 1415	81	55	43	98	50	0	0
L. delbrueckii ssp. *delbrueckii* CRL 1413	58	35	8	82	80	64	25

Note: nd, not determined.

centrations of NaF evaluated on the growth of lactobacilli isolated both from caries-free and caries subjects are presented. The results are concordant with those summarized in Table 1. From this table, we can conclude that each strain of microorganisms must be considered individually, without getting into general conclusions. The different concentrations evaluated produced significantly different values of the maximal OD, but these changes depended on the species of lactobacilli tested, as shown in Table 1. The percentage of inhibition of the growth of lactobacilli by 1 mmol·L^{-1} NaF was between 5% and 46%, at 5 mmol·L^{-1} between 13% and 65%, and at 20 mmol·L^{-1} between 57% and 84%.

The inhibition of the growth of *L. plantarum* CRL 1356, *L. salivarius* CRL 1355, and *L. delbrueckii* ssp. *delbrueckii* CRL 1360 and CRL 1415 was directly proportional to the increased concentration of NaF (also shown in Table 1, parameter *A*). The growth of *L. salivarius* CRL 1414 showed a higher inhibition at 1 mmol·L^{-1} NaF than at 5 mmol·L^{-1} NaF. Even though the maximal OD of *L. delbrueckii* ssp. *delbrueckii* CRL 1360 was close to that of *L. delbrueckii* ssp. *delbrueckii* CRL 1416, the inhibition of the growth of the last strain was not directly proportional to the concentration of the agent: only 5 and 20 mmol·L^{-1} NaF produced a significant inhibition.

Table 1 shows the statistical significance of the influence of the concentrations of NaF when comparing the growth parameters obtained with each concentration and those of the control curve.

The maximal OD, expressed as *A*, showed statistically significant differences at 5 and 20 mmol·L^{-1} NaF compared with control curves in all of the strains assayed. The 1 mmol·L^{-1} concentration did not show statistically significant differences in *A* for the CRL 1416 and CRL 1413 strains. The growth rate parameter showed differences for all the strains, except for *L. salivarius* CRL1355 only at 20 mmol·L^{-1} NaF. In the parameter lag phase duration, only 2 NaF concentrations showed significant differences in strains CRL 1415 and CRL 1416. The length of the lag phase showed no statistically significant differences for the smaller concentration of NaF for all of the strains evaluated.

Fig. 2. Effect of different concentrations of CHX on lactobacilli of subjects caries free and subjects with caries.

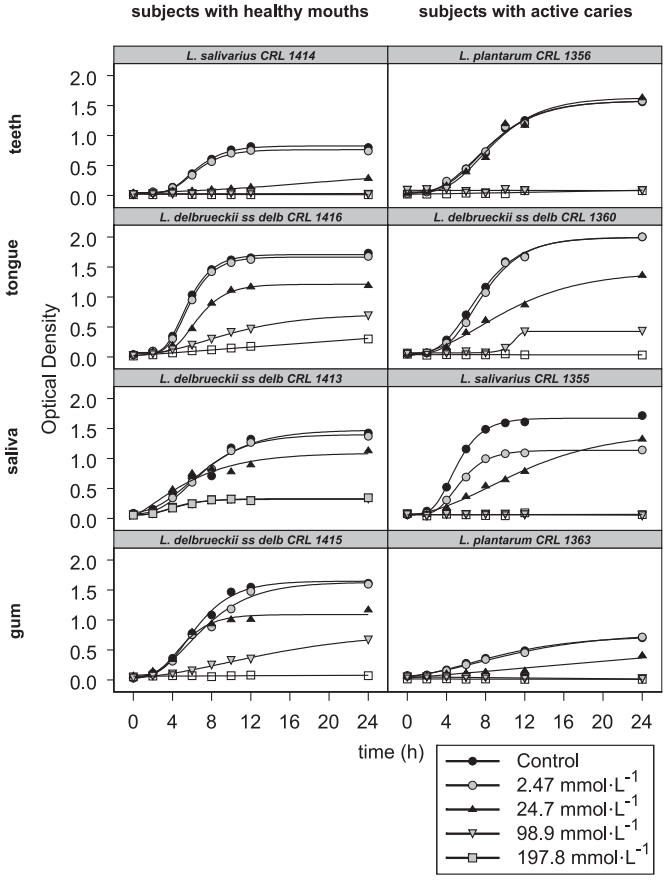


Table 3. Estimation of the growth parameters by application of the Gompertz model to the growth of oral lactobacilli and the inhibitory effect of CHX at different concentrations.

Strain	Concentration (mmol·L^{-1})	N_0	A ± SE	μ ± SE	λ ± SE
L. delbrueckii ssp. *delbrueckii* CRL 1360	Control	0.05	1.94±0.18	0.24±0.04	3.32±0.62
	2.47	0.063	1.93±0.26	0.25±0.05	3.93±0.63
	24.7	0.001	1.42±0.6	0.09±0.02	2.15±0.74
	98.8	0.07	0.35±0.004	0.26±0.002	9.72±0.02
	197.8	0.03	0.002±0.004*	0.08±0.06	2.02±1.10*
L. plantarum CRL 1363	Control	0.004	0.73±0.10	0.04±0.007	0.23±0.86
	2.47	0.016	0.76±0.16	0.04±0.009	0.45±1.24
	24.7	0.015	0.74±0.43	0.013±0.002*	0.93±2.53
	98.8	0.04	0.001±0.001*	0.10±0.10*	1.92±0.79*
	197.8	0.01	0.001±0.001*	0.07±0.31*	1.76±0.48*
L. salivarius CRL 1355	Control	0.07	1.59±0.06	0.33±0.02	2.62±0.39
	2.47	0.06	1.06±0.04*	0.22±0.03*	2.96±0.59
	24.7	0.0043	1.44±0.28*	0.08±0.01*	1.87±0.78
	98.8	0.05	0.01±0.007*	0.09±0.07*	2.11±2.55*
	197.8	0.06	0.003±0.01*	0.08±0.29*	2.03±0.98
L. plantarum CRL 1356	Control	0.05	1.51±0.85	0.16±0.03	3.72±0.73
	2.47	0.04	1.54±0.75	0.16±0.06	3.30±0.99
	24.7	0.04	1.58±0.71	0.17±0.18	4.05±1.06
	98.8	0.08	0.001±0.003*	0.10±0.39	1.92±2.05
	197.8	0.02	0.003±0.01*	0.35±0.03	2.12±4.85
L. delbrueckii ssp. *delbrueckii* CRL 1416	Control	0.06	1.64±0.16	0.35±0.03	3.22±0.36
	2.47	0.06	1.59±0.09	0.34±0.02	3.41±0.37
	24.7	0.07	1.13±0.66*	0.22±0.02*	4.05±0.56*
	98.8	0.01	0.70±0.12*	0.05±0.004*	2.13±0.74
	197.8	0.001	0.34±0.07*	0.01±0.001*	0.15±0.38
L. delbrueckii ssp. *delbrueckii* CRL 1415	Control	0.05	1.58±0.21	0.22±0.05	2.79±0.96
	2.47	0.011	1.61±0.29	0.17±0.03*	2.16±1.13
	24.7	0.07	1.61±0.29*	0.17±0.03	2.16±1.13
	98.8	0.01	0.79±0.47*	0.03±0.01*	1.47±1.35
	197.8	0.05	0.79±0.47*	0.10±0.09	1.84±1.84
L. salivarius CRL 1414	Control	0.04	0.78±0.11	0.14±0.05	3.64±0.80
	2.47	0.05	0.71±0.06*	0.13±0.07	3.75±0.64
	24.7	0.01	0.69±0.69	0.01±0.002*	3.88±2.67
	98.8	0.02	0.01±0.003*	0.08±0.006*	3.88±2.67*
	197.8	0.01	0.008±0.006*	0.08±0.34*	2.00±1.98*
L. delbrueckii ssp. *delbrueckii* CRL 1413	Control	0.08	1.39±0.91	0.13±0.04	1.99±1.58
	2.47	0.06	1.33±0.27	0.15±0.02	2.52±0.83
	24.7	0.001	1.03±0.14*	0.11±0.05	0.25±0.97
	98.8	0.06	0.25±0.05*	0.05±0.12*	1.70±1.52
	197.8	0.05	0.27±0.03*	0.04±0.01*	1.57±1.04

Note: N_0, initial data; A, incremented OD between D_0 and OD_{max}; μ, maximum growth rate (h^{-1}); λ, duration time of lag phase.
*Effect statistically significant.

The multivariate analysis with the nonlinear mixed-effects model combining the variables concentration of NaF, health state of subjects, origin of strains, and species shows a statistically significant influence of all of these effects on the growth parameters.

The concentration of NaF has a significant effect on the maximum OD ($p < 0.0001$), growth rate ($p < 0.0001$), and lag phase ($p = 0.0015$). The health state of the subjects has a significant influence on the maximum OD ($p = 0.0183$) and growth rate ($p < 0.0001$), while the origin of the strains shows an effect on the maximum OD ($p = 0.0002$), growth rate ($p < 0.0001$), and lag phase ($p < 0.0001$). Microbial species has a significant effect on the maximum OD ($p = 0.0007$), growth rate ($p < 0.0001$), and lag phase ($p = 0.0071$).

When comparing the behavior of our wild-type strains with ATCC strains, we can conclude that at high NaF concentrations, the behavior of the CRL strains was similar to that of the ATCC strains. At lower concentrations, the strains isolated by our group show a higher resistance to NaF. The inhibitory percentages obtained, both both ATCC and CRL strains, are summarized in Table 2.

Fig. 3. Effect of NaF on *S. mutans* ATCC 25175.

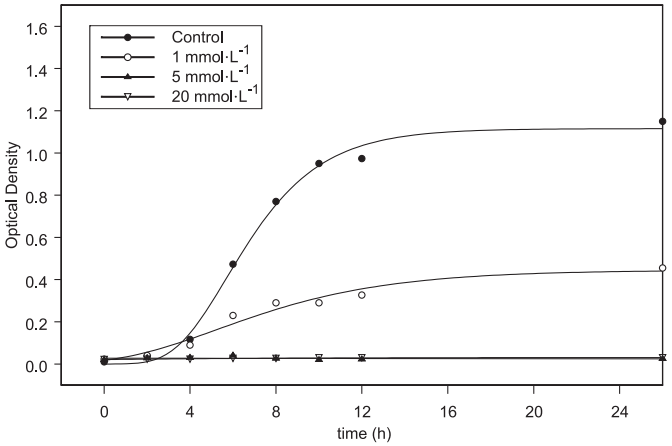
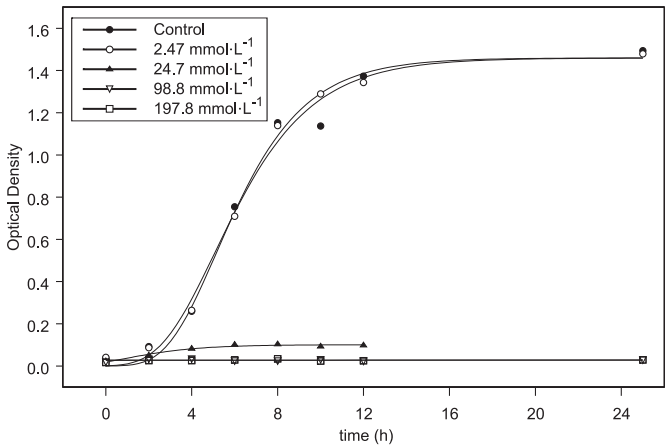


Fig. 4. Effect of CHX on *S. mutans* ATCC 25175.



Effect of CHX digluconate on the growth of oral lactobacilli

The results obtained from the nonlinear regression with the different strains are shown in Fig. 2. The behavior of the lactobacilli strains with different CHX concentrations was characteristic for each *Lactobacillus*, without the possibility of association of these results with the original place of isolation or with the type of patients from where they come. The inhibition of the growth presented a different pattern for each *Lactobacillus* according to the concentration used (Table 3).

The higher CHX concentrations evaluated (197.8 and 98.9 mmol·L^{-1}) showed a complete inhibitory effect on *L. salivarius* CRL1414, *L. salivarius* CRL 1355, and *L. plantarum* CRL 1363. *Lactobacillus plantarum* CRL 1356 did not show medium values of inhibition: it was 0% or 100%.

When comparing the growth parameters maximal OD, growth rate, and lag phase duration at each of the CHX concentrations used with the parameters of the control curve, the smallest concentrations of CHX showed no significant effect on most of the lactobacilli studied.

By using 98.8 mmol·L^{-1} CHX, the maximum OD of nearly all of the strains showed significant differences compared with the control (without the addition of CHX). The results are summarized in Table 3 where the statistically significant differences obtained among the different parameters in comparison with a control are shown.

Table 4. Estimation of the growth parameters by application of the Gompertz model to the experimental results of the growth of *S. mutans* ATCC 25175 and the inhibitory effect of NaF and CHX at different concentrations.

Concentration (mmol·L^{-1})	$D_0 + A$	μ	λ
NaF			
0	1.115173	0.169017	3.288992
1	0.444792	0.036485	0.778765
5	0.02779	0.783433	0
20	0.027778	0.052777	1.468146
CHX			
0	1.459128	0.210932	2.529293
2.47	1.459621	0.23255	2.843262
24.7	0.099876	0.02536	0
98.8	0.027143	0.301671	1.204544
197.8	0.027833	0.113984	1.668833

Note: A, incremented OD between D_0 and OD_{max}; μ, maximum growth rate (h^{-1}); λ, duration time of lag phase.

The multivariate analysis obtained from the nonlinear mixed model indicated that the concentration of CHX exerts a statistically significant effect on the values of maximum OD ($p < 0.0001$) and growth rate ($p < 0.0001$). The health state only affects the maximum OD ($p = 0.0001$), the same as the origin ($p = 0.0027$). Species had a significant influence on the maximum OD ($p < 0.0001$) and growth rate ($p = 0.0008$).

The experiments performed with ATCC strains showed very wide behavior; *L. plantarum* CRL 1356 and CRL 1363 have inhibition patterns similar to those of *L. plantarum* ATCC 14917. The *L. salivarius* from ATCC or CRL also showed similar behavior, but *L. delbrueckii* ssp. *delbrueckii* CRL 1416 and CRL 1413 were more resistant to CHX than the type strain ATCC 9649. The results are summarized in Table 2.

Effect of NaF and CHX on *S. mutans* ATCC 25175 growth

The effects of the 2 substances NaF and CHX on the growth of *S. mutans* are shown in Figs. 3 and 4. The degree of inhibition with NaF on the growth of *S. mutans* was higher compared with lactobacilli, being 60% at 1 mmol·L^{-1} NaF and 97% at 5 and 20 mmol·L^{-1} NaF. The growth parameters are shown in Table 4.

In the experiments with CHX, the degree of inhibition was complete at the higher concentrations, while at the lower concentrations studied, there was no inhibition. The growth parameters are shown in Table 4.

Discussion

Prophylaxes and preventive measures are applied with higher frequency to eliminate cariogenic microorganisms. The effects of CHX and NaF have been studied on the strep-

tococci genera, more specifically in the mutans group (Wilson et al. 1998; Yoshihara et al. 2001; de Soet et al. 2002). Topical fluoride is used in fluoridated mouthwash at different concentrations, toothpastes, topical fluorides for professional application, autoapplication gels, and varnishes fluorides (Yoshihara et al. 2001). The frequent topical applications of fluorides at low concentrations inhibit the demineralization and favor the remineralization of the enamel by enough levels of this ion in all cases when the pH decreases (van Houte 1994; Featherstone 1994).

To further study the behavior of different species of oral lactobacilli in the presence of these compounds, the present work was designed to evaluate their effect at different concentrations on the growth of selected microorganisms. The results showed that the degree of inhibition of the growth by different concentrations of NaF and CHX is very heterogeneous and is not dependent on the strain or the original places of isolation. For the laboratory experiments, NaF was evaluated at a wide range of concentrations between 1 and 20 mmol·L^{-1}. The published papers showed that the higher concentrations of fluoride exert a germicide effect, considering that they are higher than 5 mmol·L^{-1} (Balzar Ekenbäck et al. 2001). The results of this paper show that the higher degree of inhibition was produced with 20 mmol·L^{-1} NaF. If the inhibition percentage of this compound is compared with the effect on *S. mutans*, this bacterium is more sensitive to the action of fluoride than lactobacilli, experiments included also in the present paper. The ATCC reference strains were shown to be more sensitive to the lower NaF concentrations than the strains isolated in our laboratory. There is a higher resistance of our strains to NaF, these results being remarkable, since the strains of lactobacilli potentially probiotic should be more resistant to higher concentrations of NaF, which are germicide for the cariogenic *S. mutans* strains. NaF concentrations of 20 and 5 mmol·L^{-1} inhibit the growth of *S. mutans* at 97%, while 1 mmol·L^{-1} inhibits at 60%. The concentrations of NaF administered in rinses and gels are around 0.05%, equivalent to 12 mmol·L^{-1}. Dogan et al. (2002) showed that 1–2 mmol·L^{-1} NaF reduces the viability of *S. sobrinus* significantly.

In the experiments with CHX, although a statistically significant inhibition of lactobacilli growth was observed when using concentrations of 197.8 and 97.8 mmol·L^{-1}, the effect was smaller at 24.4 and 2.44 mmol·L^{-1}. According to the results reported by Cleghorn and Bowden (1989), lactobacilli were sensitive to smaller concentrations (0.002–0.2 mmol·L^{-1}) of CHX than those reported in the present work. These results agree with those of Bothelho (2000) who reported that CHX at 0.0005–0.016 mmol·L^{-1} was inhibitory for lactobacilli and streptococci. The CHX concentration used for clinic applications is 0.12%, equivalent to 1978 mmol·L^{-1}, a concentration 1000 times higher than the one used in this work. The sensitivity of different *Lactobacillus* species varies; *L. casei* was relatively resistant (Cleghorn and Bowden 1989), *L. delbrueckii* ssp. *delbrueckii* CRL 1416 and CRL 1413 were shown to be more resistant to CHX than the ATCC 9649 strain.

The growth parameters were calculated performing a nonlinear regression with the Gompertz model. This method also allows comparison of the parameters of different growth curves and the degrees of inhibition of the growth. The nonlinear mixed model has the advantage of the evaluation of the multivariate effects; both methods are used to make statistical inferences about the possible effects of the influences of different experimental conditions.

The application of a statistical model to analyze the growth of microorganisms is currently being used and is largely applied in the field of predictive microbiology to deal with contamination and storage of foods. The Gompertz model has been much applied to data obtained on growth measured as OD of different lactobacilli and streptococci (Zwietering 1990; McClure et al. 1994*a*, 1994*b*).

The results presented in the present paper allow more rapid formulation of several conclusions on the behavior of different lactobacilli compared with cariogenic streptococci as a new approach to evaluate the resistance of potentially probiotic microorganisms to be applied in patients that frequently use mouth rinses.

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