Journal of Oral Science, Vol. 45, No. 1, 1-9, 2003

Original

Evaluation and comparison of lactobacilli characteristics in the mouths of patients with or without cavities

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(Received 6 January 2002 and accepted 22 January 2003)

Abstract: Lactobacilli were considered the prime cariogenic microorganisms until phylogenetic related bacteria, mutans streptococci, were associated with caries. Today, lactobacilli are still considered a factor in determining the predisposition to develop cavities. As a first step in colonization, microorganisms adhere to oral tissues. Based on this fact, the purpose of our study was to determine if there was a statistical association or difference related to the state of oral health with the surface characteristics of lactobacilli. Patients were classified as caries-free and caries-active. Interviews were performed to establish the nutritional and oral hygiene habits. The previously reported characteristics of isolated lactobacilli's quantification and association to dental tissues were determined.

Although the nutritional habits for caries-free and caries-active patients were similar, the patients' oral hygiene and dental care determined differences in risk indices. The number of lactobacilli was statistically lower in saliva of subjects with good oral health. Certain species of lactobacilli could not be associated to specific areas of the mouth, although some species could be localized. Lactobacilli from caries active (CA) subjects showed a greater ability to adhere to hydrophobic substances, had a greater salt agglutination property, and showed lower production of inhibitory substances. Lactobacilli from caries free (CF) subjects were better able to inhibit oral, potentially pathogenic, microorganisms. These studies prove that preliminary differences between oral lactobacilli in CF and CA patients exist. Non-specific and specific adhesion mechanisms in bacteria should be further demonstrated. (J. Oral Sci. 45, 1-9, 2003)

Key words: Lactobacilli; adhesion, properties, oral health.

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Introduction

Lactic acid bacteria are described as probiotic microorganisms in a variety of mucosal sites (1). In the oral cavity, these lactic acid bacteria were originally considered as members of the cariogenic process, because they produce acid and are aciduric (2-3). In the 1960's it was believed that *Streptococcus mutans* also contributed to the cariogenic process. Today, however, few specific organisms of the dental plaque are considered to play a special role in the development of cavities (5). Other studies have been carried out to consider the properties of saliva in relation to the progression of cavities (6). Those studies have shown that the salivary flow rate is an important factor. Lactobacilli generally constitute a low proportion of the plaque microbiota. It has been suggested, however, that their role is significant in the progression of cavities rather than in their initiation (7).

Some reports have shown that there exists a clear association between the state of the oral health and the microflora present in the mouth. It has recently been demonstrated that *S. mutans* strains isolated from the saliva of caries-free individuals cause a greater stimulation of neutrophils than those collected from root caries-active patients, suggesting that *S. mutans* develop a biological

mechanism to circumvent neutrophil recognition (8). The adsorption and adhesion to hydroxyapatite in *in vitro* assays of this microorganism was also demonstrated (9). Lactobacilli are also present in caries-free mouths, but they have not been described as extensively as *S. mutans*. In non-healthy mouths they are present in high numbers in the saliva, the dorsum of tongue, the vestibular mucosa, the hard palate, and in smaller amounts in the teeth (10).

The adhesion mechanisms of bacteria of the genus lactobacillus in the oral cavity have not been widely studied with the exception of their coaggregation capabilities (11). Only recently, the *in vitro* adhesion of two lactobacillus strains isolated from yogurt to enamel have been studied (12). *L. acidophilus* and *L. casei* can adhere to enamel chips either with or without a salivary film. However, it should be noted that salivary and inter-proximal plaque samples are not rich in lactobacilli after the consumption of yogurt.

In previous reports, lactobacilli from caries-free (CF) (13) and caries-active (CA) patients (14,15) were isolated, identified and characterized. The adhesion-predictive characteristics of lactobacilli to tissues of the oral cavity were also studied in *in vitro* experiments. Hydrophobicity, surface charges, salt aggregation and hemmagglutination properties had been described for the isolated lactobacilli. In the present work, the previously reported results and new information concerning the patients' characteristics and the areas of the mouth where lactobacilli were originally isolated (dental surface, total saliva, dorsum of tongue and gingiva) are analyzed, associated statistically and reported. The screening performed in all of the lactobacilli strains to evaluate the levels of inhibitory substances is also reported in this paper. With all of this available information, it is possible to point out differences between the lactobacilli characteristics and their interactions in the oral cavity of CF and CA subjects.

Materials and Methods

Population

This study included 44 CF patients and 22 CA subjects living in Tucumán, Argentina. The consent of the subjects and the approval of the Institutional Ethics Committee were obtained before any testing began. In those patients, lactobacilli were isolated from 22 of the 44 subjects classified as caries-free (CF) (13) and from 17 of the 22 caries-active (CA) patients (15). Halogen light and X-rays were employed to classify any doubtful diagnoses of caries. An experienced dentist examined all patients before they were included in the experiment.

The following indexes were obtained in the patients (according to World Health Organization criteria):

Caries Free: DMFT, dmft, DMFS and dmfs ≤ 6, the components being D, M, d and m 0.

F ≤ 6; Gingival Index (GI) ≤ 0.35 and Plaque Index ≤ 0.70. These patients can be considered as moderate caries risk, because they had shown caries in the past, with less than 6 fillings at the time of the study. In six patients the presence of white spots was detected.

Caries Active: DMFT and DMFS > 6, D and d being ≥ 5; Gingival Index (GI) > 0.40 and Plaque Index > 0.70. The white spots were considered as caries, but they were recorded for further associations. These patients did not present any white spots. The plaque and gingival index are a diagnosis complement, because they show a higher risk of caries if they are higher than 1.

The salivary flux and pH of non-stimulated saliva were also recorded.

The patients were not grouped by age or by sex, but only by the healthy or non-healthy state of their oral cavity, because the main objective of this study was to study the adhesive characteristics of lactobacilli, not to perform epidemiological studies.

Interview

Subjects' diet history, oral habits and socio-economical level were determined in an interview of 1 h. Questionnaires were based on closed (yes-no) answers and included the caries risk factors (16). The use of fluoride agents, the frequency of carbohydrate intake, and the buccal hygiene were considered. Other aspects recorded were the patients' nutritional habits, exposure to the sun and medical and biochemical history. Any patient that had consumed hormones or antibiotics in the last 20 days, smoked, or had orthodontic treatment or any buccal inflammation were excluded from this study.

Sample Collection and Conservation

The manner in which samples were colleted and conserved has been described in previous papers. Lactobacilli were isolated, identified, and their surfaces characterized (13-15) using a process of hydrophobic partition with hexadecane, xylene and toluene, in addition to basic and acidic surface characteristics with chloroform and ethyl acetate, respectively. ABO human erythrocyte haemagglutination, aggregation in the presence of salt (0.2-2 M ammonium sulfate) and auto aggregation in microplates were also performed. Quantification of the microorganisms was based upon the number of lactobacilli isolated per cm^2 of the tongue and gums, per dental piece (teeth) and per ml of unstimulated saliva secreted in 1 min. In each patient the area of the tongue and gum (in cm^2) where the samples were taken was established before taking the sample.

Inhibitory Substance Production

Lactobacilli stored at - 20°C were subcultured three times in LAPTg broth (17) at 37°C. The microorganisms were collected using centrifugation (2000 × g for 30 minutes). The supernatants were separated and sterilized by filtration (Millipore membranes, 0.22 µm). At the same time, LAPTg plates were prepared containing 10^6-10^7 CFU/ml of the following organisms: *S. mutans* ATCC (American Type Culture Collection) 25175, *S. sanguis* ATCC 15300, *S. anginosus* 246/93, *S. sanguis II* 187/93, *S. anginosus milleri* 1796, *S. salivarius* 332/93, *S. mitis* ATCC 49456, *S oralis* ATCC 35037. The non-lactobacilli strains were provided by the Instituto Malbrán, Argentina. In the agar plates, 4 mm holes were punched, in which the lactobacilli supernatants were added (25 µl). The plates were left to settle for 5 h to allow for supernatant diffusion, and later incubated for 24 h at 37°C. After this process, the inhibition halos were measured. To determine the nature of the inhibitory substances, the supernatants were neutralized with 2N NaOH and treated with catalase (1000 UI/ml) for 1 h at 37°C.

Statistic Evaluation of Lactobacilli Quantification and Characterization

The Spearman correlation (18) coefficient was used to establish associations of variables between groups. The statistical analyses of the differences between lactobacilli isolated from CF and CA patients were performed by the ANOVA (18) and HSD Tukey test for each of the surface characteristics considered. The non-parametric test of Kruskal-Wallis (18) was applied to compare bacterial quantification of both groups of patients and the χ^2 test (18) to calculate the signification of lactobacilli metabolic group by analysis of the contingence tables. The software Minitab R12.23 was used for calculations.

Results

Lactobacilli Isolation and Quantification

A total of 145 microaerophilic strains of lactobacilli were isolated. Their identification and some of their characteristics were stated in previous papers (13,14,15). Lactobacilli were generally not obtained from all four sites in our subjects. This occurred in only one CF and three CA patients. Samples were taken from 44 CF patients, and from 22 CA patients. In 50% of the CF subjects, and in 22.7% of the CA subjects, no lactobacilli were isolated. There was not ample statistical evidence to specify a predominant metabolic group in CF or CA patients, as was shown by the χ^2 test.

Table 1 shows mean values of Log CFU per measuring unit. The HSD Tukey test indicated that there were significant differences ($P < 0.001$) between the mean values of the CA patients' saliva variables and the other mean values, except those from the CA patients' gingiva.

Even though none of the lactobacilli species has a predominant isolation frequency in the patients, *L. fermentum* was isolated from 12 patients, and of these only two were isolated in CA. The following species appeared with almost the same frequency in both groups of patients: *L. plantarum* in 6 CA patients and 7 CF patients, *L. paracasei* ssp *paracasei* (2 and 4) and *L rhamnosus* (6 and 5). The other lactobacilli species showed a lower than 10% frequency in appearance in both CA as in CF.

Lactobacilli Properties

A) Degree of Hydrophobicity.

General Results. The results of the hydrophobicity percentage of the strains studied are presented in the matrix plot of Fig.1A and B of CF and CA patients, respectively. The hydrophobicity of the strains was tested with three organic solvents: hexadecane, xylene and toluene. It can be concluded that the majority of lactobacilli (regardless of the area from which they were isolated) from CF individuals show a high degree of hydrophobicity with at least one of the three employed solvents, while the strains that had been isolated from CA patients showed much lower values.

A statistically significant correlation ($P < 0.01$) was observed between the variables of hexadecane, xylene

Table 1 Quantification of lactobacilli from the oral cavity of patients. Means, standard deviation and sample number of Lactobacilli from different areas of the mouth calculated from n patients(1)

Patients	Area	Mean	Std. Dev.	n
CA	Saliva	2.3^a	0.86	17
CA	Teeth	1.0^b	0.99	13
CA	Tongue	1.3^b	0.87	23
CA	Gum	1.3^{ab}	1.00	7
CF	Saliva	0.85^b	0.37	24
CF	Teeth	0.95^b	0.64	15
CF	Tongue	0.58^b	0.61	36
CF	Gum	0.76^b	0.55	10

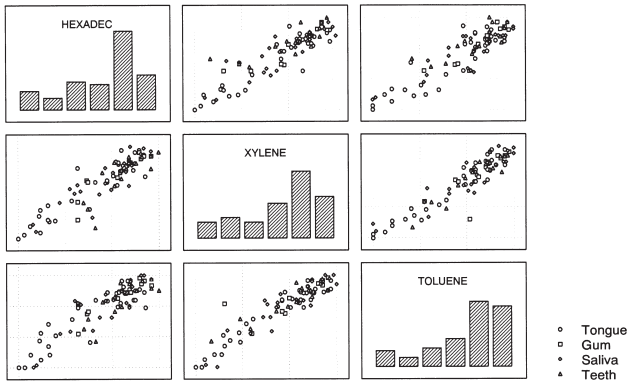
CA: caries active patients. CF: caries free patients.
(1) The results are expressed as follows: Saliva: Log CFU/ml/min; Teeth: Log CFU/dental piece; Tongue and Gum: Log CFU/cm.
(a),(b)Different superscript letters between two mean values indicate statistically significant differences with $P < 0.01$

and toluene, even in the lactobacilli from both CF and CA patients. The Spearman correlation coefficient "r"varied between 0.78 and 0.98 for CA patients and 0.76 and 0.94 for CF subjects. In the histograms and in the dispersion diagram shown in Fig. 1, the dots, which stand for solvent values in microorganisms, are concentrated higher up on the graph in CF patients, while the dot concentrations for CA patients represent low hydrofobicity values.

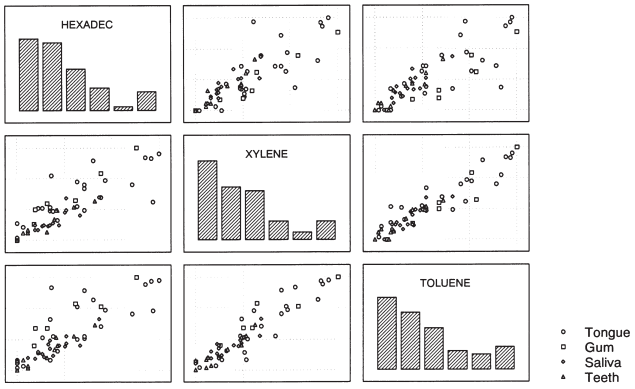
Definition of Areas Results. The differences in the degree of hydrophobicity of the isolated lactobacilli were quantified by an ANOVA test of two factors: the buccal state (CA and CF) and the area of the mouth (teeth, saliva, tongue and gums). One ANOVA value was calculated for the hexadecane, toluene and xylene variables. The results are expressed in Table 2. This test demonstrates that the behavior of microorganisms with hexadecane, xylene and toluene depends on both of the factors considered. Even in saliva, teeth, tongue and gingival, the isolated lactobacilli were more hydrophobic in CF than in CA patients. The hydrophobicity of CF and CA patients was significantly higher in the teeth and saliva than in the tongue and gums, as indicated by the different letters in the superscript. Fig. 2 A summarizes the interactions of the solvents employed; it shows the behavior of the experimental values of these three characteristics obtained in lactobacilli from the different areas of CA and CF. It can be therefore presumed that lactobacilli which are isolated from CA patients will present lower values of hydrophobicity, while those which are isolated from CF patients will show relatively higher hydrophobicity values.

B) Acid and Basic Characteristics. The tendency that lactobacilli surfaces have to be electrically charged was also analyzed. Ethyl acetate was used for cases of acidity, and chloroform for basicity. One ANOVA was calculated for both chloroform and ethyl acetate. Table 3 shows that the behavior of microorganisms with both solvents depended on the oral site of isolation. In all the areas of the mouth, except for the tongue, there were big differences between the values obtained from CF patients and the CA patients (indicated by all different letters in the superscripts). As shown in Fig. 2 B, the behavior of lactobacilli towards acetate and chloroform was the same as that towards

Fig.1. Matrix plot of the relationships between hexadecane, xylene and toluene partition of lactobacilli isolated from caries free and caries active patients.



a) Caries Free



b) Caries Active

Table 2. Average values of partition percentage of hexadecane, xylene and toluene from lactobacilli isolated from saliva, teeth, tongue and gums of CF and CA patients

Hexadecane	**Saliva**	**Teeth**	**Tongue**	**Gum**
Caries Active	22.6 ^{a}	18.5 ^{ad}	41.1 ^{ac}	31.1 ^{ac}
Caries Free	54.4 ^{bc}	72.1 ^{b}	49.1 ^{bcd}	63.7 ^{bc}
Xylene				
Caries Active	19.5 ^{a}	16.0 ^{a}	43.0 ^{ab}	40.6 ^{ab}
Caries Free	58.5 ^{b}	67.3 ^{b}	51.4 ^{b}	65.7 ^{b}
Toluene				
Caries Active	21.1 ^{a}	16.6 ^{a}	42.1 ^{ab}	47.4 ^{ab}
Caries Free	59.7 ^{b}	71.3 ^{b}	53.1 ^{b}	70 ^{b}

$^{(a),(b),(c),(d)}$Different letters between two mean values indicate differences statistically significant with $P < 0.01$

Table 3 Mean values of chloroform and ethyl acetate partition of lactobacilli isolated in different areas of CA and CF patients

Chloroform	**Saliva**	**Teeth**	**Tongue**	**Gum**
Caries Active	36.4 ^{a}	20.9 ^{a}	55.4 ^{ac}	45.3 ^{ac}
Caries Free	71.5 ^{bc}	75.3 ^{bc}	56.8 ^{ab}	79.2 ^{bc}
Ethyl Acetate				
Caries Active	22.3 ^{a}	20.4 ^{a}	48.6 ^{ac}	31.0 ^{ac}
Caries Free	54.0 ^{ab}	68.8 ^{bc}	54.3 ^{ab}	67.7 ^{bc}

$^{(a),(b),(c)}$Different letters between two mean values indicate statistically significant differences with $P < 0.01$

hexadecane, xylene and toluene. Partition degrees are significantly higher in CF patients than in CA patients. Differences between CF and CA were also higher in teeth and saliva.

Nevertheless, a very low correlation between acetate and chloroform has been observed. These two variables and hydrophobicity also show relatively low correlation values. There were some lineal relationships that were statistically significant in the tongue and gingiva, but they were not associated with the buccal state.

C) Haemmaglutination. A low percentage of the isolated strains demonstrated the haemagglutination characteristic (13,14,15). Only 29% of the lactobacilli isolated from CF mouths and 31% from CA mouths agglutinated human red cells. However, all lactobacilli from CF subjects haemmagglutinated the three blood groups. Only 6 strains from CA reacted with the ABO groups, but 3 lactobacilli reacted with only one or two red blood cell type. In neither group A nor B did lactobacilli haemagglutination present an evident correlation with the buccal state or the area of the mouth from which the microorganisms were originally isolated. Due to the presence of this characteristic in only a small number of strains, it was not possible to obtain a correlation with the metabolic groups of lactobacilli. The results of these experiments are shown in Fig. 3.

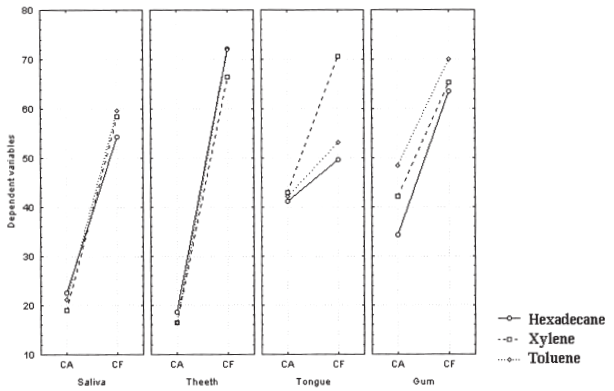


Fig. 2 A Influence of the buccal state and the origin of lactobacilli isolation on the hydrophobicity and surface characteristics of microorganisms with hexadecane, xylene and toluene.

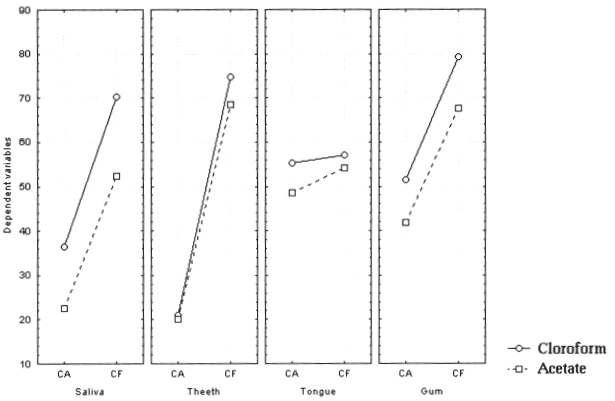


Fig 2 B Influence of the buccal state and the origin of lactobacilli isolation on the hydrophobicity and surface characteristics of microorganisms with acetate and cloroform.

D) Salt aggregation. This property was observed for 34 lactobacilli of CF subjects, and some of them even aggregated with 2 M, the highest salt concentration (13-15). Only 13 lactobacilli strains from CA patients aggregated. There was a significant positive correlation (close to r = 0.30) between the hydrophobicity and haemagglutination results in CF subjects, but no correlation was observed for the CA patients.

E) Auto aggregation. Lactobacilli auto aggregation was mainly observed in CA mouths (39/60) with much less being observed in CF patients (33/85). Even though from the 72 microorganisms showing auto-aggregation properties, 39 were isolated from of CA mouths, the χ^2 test did not indicate that this difference was statistically significant.

It was demonstrated that the associations between auto aggregation and hydrophobicity in organic solvents are higher in lactobacilli from CF than those from CA, although the Spearman correlation test gave low values, between 0.25 and 0.40, which indicated very weak associations. In

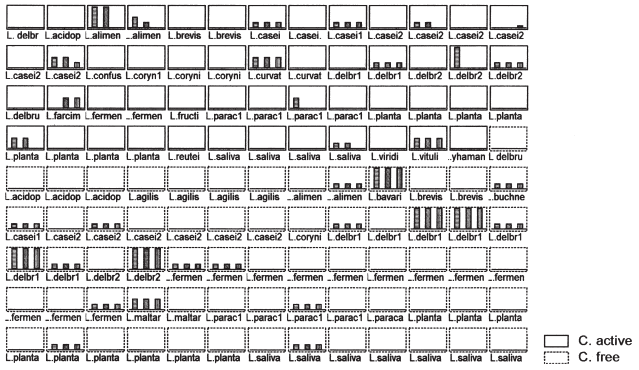


Fig. 3. Hemmaglutination produced by lactobacilli isolated from healthy or non-healthy subjects. Each box represents one isolated microorganism.
The height of the columns represents the hemmaglutination titration (from 1 to 8).

respect to haemmagglutination, statistically significant lineal relationships were obtained in CA and CF patients but the correlation coefficient values were higher for the lactobacilli from CF mouths. The relation between auto aggregation and salt aggregation was also higher in CF patients than in CA patients.

F) Inhibitory Substances Production. The supernatants of lactobacilli isolated from healthy patients showed a very low degree of inhibition. They mainly inhibited *S. mutans*. The majority of the lactobacilli producing inhibitory substances were those isolated from the tongue (5/36 strains) and from saliva (4/24). However, the lactobacilli isolated from CA subjects showed a higher production of inhibitory substances. The percentages of strains producing inhibitory substances were higher in the group of CA patients, with a wider spectra of inhibition, because the strains inhibited were the following: *S. mutans, S. sanguis I, S. sanguis II, S. anginosus, S. anginosus milleri, S. salivarius, S. oralis,* and *S. mitis*. The number of strains producing inhibitory substances was as follows: 6/13 in the teeth, 2/7 in the gingiva, 7/23 in tongue and 9/17 in saliva.

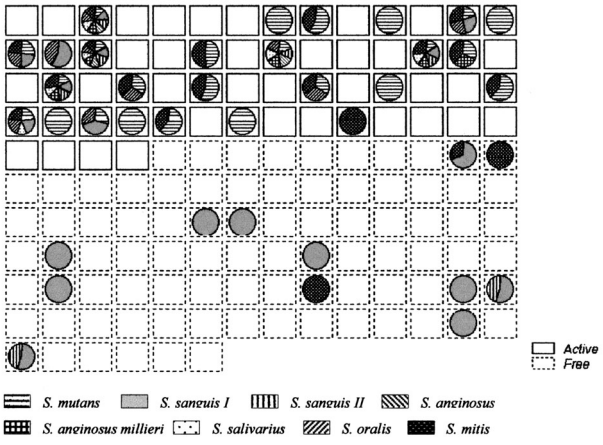


Fig 4. Inhibitory substances produced by lactobacilli isolated from healthy and non-healthy patients. Each box represents one isolated lactobacilli, and the circle represents the production of inhibitory substances. The symbols inside the circle indicate the microorganisms inhibited, each one of which is represented by a different symbol. (*S. mutans*, *S. sanguis I*, *S. sanguis II*, *S. anginosus*, *S. anginosus milleri*, *S. salivarius*, *S. oralis*, *S. mitis*).
The magnitude of inhibition is only represented when two microorganisms were inhibited by one strain of lactobacilli. When more than two microorganisms were inhibited, the symbols indicate which of them was inhibited, without the magnitude of inhibition.

The results obtained are shown in Fig 4. Each square represents one lactobacillus strain, uncut lines designate CA values, while dotted lines represent values from CF. The circumferences represent the inhibition of lactobacilli against the different microorganisms, as indicated by different symbols. The area of the circle shows the degree of inhibition only when two microorganisms were inhibited. When more than two bacteria were inhibited, the symbols represent the different microorganisms inhibited, but not the degree of inhibition.

Discussion

The oral cavity is an environment colonized by microorganisms that maintain its ecological balance, or converts to a state of illness (19). The adherence of bacteria is considered the first step in colonizing an environment, and has been suggested by Piette and Idziak (20) as the result of specific and non-specific mechanisms. Non-specific binding involves electrostatic or hydrophobic interactions of lower affinity than those interactions in specific bindings. The adhesion function has not been thoroughly described for the lactobacilli of the mouth. Nor have the binding interactions with other groups of oral bacteria been studied. The present paper evaluates the hypothesis that lactobacilli isolated from different areas of the oral cavity of CF or CA subjects have different surface characteristics. To perform some studies related to the ecological role of bacteria and their function in ecosystems in which they dominate, there are some applicable *in vitro* methodologies (9,21). There are not many experimental models that allow the study of those types of interactions. Some techniques are used in the laboratory to predict surface characteristics, (21,22) and also to test if there is some type of inhibition of pathogenic microorganisms (23). Some of those techniques were applied by our research group.

The first part of the study was designed to isolate lactobacilli from different areas of the oral cavity, from two clearly defined types of patients, those with and those without cavities: caries free and caries active (13,14). Our main interest was to establish the differences between lactobacilli populations or species in both groups of patients, not in performing epidemiological studies. The phenotypic identification of the lactobacilli isolated from the four areas of the CF and CA patients was reported (13,14). The low proportion of lactobacilli isolation in both types of patients can be attributed to the method of isolation, because only microareophilic lactobacilli were recovered, but not those strictly anaerobic. The only association obtained in the present paper is that a high number of patients that were CF had *L. fermentum* in at least one of

the studied areas of the mouth. The predominant species, or their heterogeneity depends on a number of variables, mainly those related with the food intake, and also with the ecological environment. Smith et al. (24) reported the isolation of lactobacilli in human dental caries and saliva, the species present, the production of ß-lactamase and the bacteriocin production of the isolated strains. The species reported by this group are different from our results, because *L. brevis* and *L. fermentum* were the most frequent microorganisms in a wide variety of strains.

Another objective was to determine the number of lactobacilli in the different areas of the oral cavity through the application of standard microbiological methods used for other applications. Then, we used the successive dilution method and the plating in selective agar for the determination of the number of microorganisms either in surface areas, or dental pieces. This quantification was not reported before by other research groups. The only method available in the commercial literature to determine the amount of lactobacilli in the mouth was the DENTOCULT® LB (Vivacare-Vivadent), which is a semi quantitative method, and did not provide the information we sought. The number of lactobacilli counted by standard methodology showed that higher numbers of lactobacilli exist in saliva of CA patients, rather than in the saliva of CF patients, as presented in Table 1, where the statistically significant differences are indicated by different superscripts.

From the patients' evaluations it can be concluded that the most influential difference between the two groups is oral hygiene, which is directly related to the frequency of the patients' flossing and brushing. Both groups had varying degrees of oral hygiene and care. All of the CF subjects regularly visit a dentist and receive regular fluoride treatments, while 80% of the CA patients had never had dental care and most do not brush their teeth daily. The χ^2 test revealed statistically significant differences in the amounts of white spots and dental deep groves ($P < 0.05$) between the CA and CF patients. A recent report by Gibson and Williams (5) suggested that brushing twice a day with a fluoride toothpaste may protect better against cavities than restricting sugary foods. Another recent paper (25) reported that a fluoride mouth rinse inhibits mutans streptococci, not affecting the levels of lactobacilli. This hypothesis is further supported by our work, for CA and CF patients that have similar diets, but very different dental habits and assistance.

The daily diet is also influential in oral health. CF patients have a more varied diet, they consume more milk and water throughout the day, not only during meals. Both type of patients have four meals a day. General medical and biochemical antecedents were not considered as selection criteria, but 80% of all of the CA subjects included in the study had a history of parasites. The objective of our research was not the selection of groups of patients by age or gender, or to test them as a group, but only to evaluate if there were differential characteristics in the lactic acid bacteria of both types of patients, as a basis for further studying their adhesion or probiotic characteristics.

Saliva pH and flow rate were in the normal range according to Yoon's (26) criteria. For CF patients the pH and rates were reported as 6.94+/-0.43 and 0.33+/-0.28 ml/min. In CA patients, the average pH was 6.99+/-0.23 and the flux rate was recorded as 0.23+/-0.14. No statistical differences were observed between the two groups in regards to these parameters.

Looking at the characteristics of the lactobacilli isolated from the two groups of patients, and trying to form groups of lactobacilli based upon these characteristics, our results showed that most of the strains isolated from CA patients had low hydrophobicity with hexadecane, xylene and toluene and only those from the tongue showed medium hydrophobicity. However 40% of the strains from CF subjects were highly hydrophobic. Further, studies should be performed to determine the degree of correlation between the level of hydrophobicity and the capability of adhesion or cavity production by lactobacilli. In addition, they generally had a positive or negative charge. Lactobacilli from CA mouths showed a low surface charge. The only reference studies in this area were performed by Rosemberg et al., (27) who reported the degree of hydrophobicity of oral streptococci. Our research group also applied this technique to predict the adhesion abilities of the lactobacilli isolated from other microenvironments (28).

The capability for haemagglutination was also tested based upon the hypothesis that there exists a similarity in the structures of epithelial mammalian receptor cells and erythrocytes, and that the presence of lectins on the bacteria surface are proportionately related (20). A low percentage of the isolated strains showed the haemagglutination property, and those that did have this characteristic were mainly strains that had been isolated from the saliva of CA patients. There have been no previous studies about oral lactobacilli and haemagglutination in the oral cavity, although this property was applied to uropathogenic microorganisms and has also been evaluated in lactobacilli that had been isolated from the vaginal tract (28,29). Our study, however, was the first to test lactobacilli isolated from the oral cavity.

Salt agglutination is a characteristic that was present in some strains from the CF subjects, but it was not statistically related to the other previously examined characteristics.

These results indicate that the structures responsible for haemmaglutination, auto agglutination and salt aggregation are quite different. However, all of them have been suggested as *in vitro* predictive adhesive characteristics. Some of the strains also showed weak auto aggregation. The capabilities of auto aggregation and co-aggregation have been suggested as beneficial or antagonistic characteristics, depending on the ecological niche under treatment (30). If the bacteria are able to co-aggregate, they can co-aggregate pathogenic microorganisms, which are later eliminated by saliva and swallowed. The other possibility is that they promote tissue colonization, therefore they are not beneficial to the host (31). The different results obtained through the use of different methods could indicate that the same receptors and chemical structures are not involved in the interaction with red blood cells, concentrated salts, or with the same bacteria.

The inhibitory capability of lactobacilli from both groups was quite different, which suggests that the microorganisms isolated from caries active patients have some special role, which should be elucidated in the future. These lactobacilli must survive in an ecological niche with some other pathogenic microorganisms, and over time, they develop the capability to produce inhibitory substances. Our results are different from those obtained by Smith et al. (24), who reported that all the lactobacilli tested, except *L. jensenii*, produce bacteriocin against at least one of the indicators strains, mostly enteropathogenic microorganisms.

Bacteria of the genus Lactobacillus are also major role players in the ecological balance of other epithelia, such as the vaginal and the intestinal tract (22,32). During the last decade, many studies have been performed to demonstrate the use of these bacteria as probiotics (1). In the oral cavity they could also act in the co-adhesion to other planktonic microorganisms, as was described by Bos et al. (30) for oral bacteria. Because this is the first report that tries to associate characteristics of lactobacilli isolated from different areas of the oral cavity from either CA or CF patients, further studies should be performed to better define the particular function that lactic acid bacteria perform in the oral cavity.

Acknowledgments

This work was partially supported by CONICET PIP 0359 and CIUNT D/128 1998/2000 grants.

We acknowledge the valuable suggestions of Birgitt Wiese from the Medizinische Hochschule, Hannover, Germany.

We also wish to thank Sally Parting-Wagner for the English revision of the manuscript.

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