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Surface properties of lactobacilli isolated from healthy subjects

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OBJECTIVE: Lactobacilli are considered cariogenic micro-organisms. As oral species of lactobacilli have not been thoroughly described, the aim of this work was to isolate and identify these organisms from teeth, tongue, saliva and gum of healthy patients and to describe some of their surface properties.
SUBJECTS: Forty-four subjects from Tucumán, Argentina, with D, d and M, m indices equal to 0.
MATERIALS AND METHODS: Samples were obtained from different areas of the oral cavity. Microorganisms were cultured in lactobacilli selected media (LBS) and identified morphologically and biochemically. Hydrophobicity was analysed by partition in organic solvents, acidity by affinity with chloroform and basicity with ethyl acetate (MATH method), aggregation and coaggregation in presence of $(NH_4)_2SO_4$, and haemagglutination with ABO erythrocytes in microplates.
RESULTS: Eighty-five lactobacilli were isolated; 29.4% were homofermenter, 44.7% facultative heterofermenter and 25.9% obligate heterofermenter. Predominant species were *L. fermentum, L. plantarum, L. salivarius*, and *L. rhamnosus*. Most of the strains showed moderate to high hydrophobicity and demonstrated high acid and basic surface charges with almost 40% showing salt aggregation. Few strains haemagglutinated.
CONCLUSIONS: A variety of *Lactobacillus* species were isolated from healthy mouths, some of whom showed adhesion-related properties such as high hydrophobicity and charged surfaces. Probable mechanisms related to the ecological behaviour of lactobacilli in the oral cavity are discussed.
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Keywords: lactobacilli; oral health; surface characteristics; adhesion

Introduction

Adhesive interactions are a prerequisite for species to become part of an oral bio-film and several mechanisms and factors involved in oral pathogen adhesion have been described (Prakobphol *et al*, 1995). Adhesion properties attributed to lactobacilli have not been described yet, and it has never been demonstrated whether they can invade tissues or serve as receptors for other bacteria. There exist, however, many other studies on lactobacilli in relation to maintenance of the ecological balance on other epithelia (Redondo-López *et al*, 1990; Nader-Macías *et al*, 1996). Control of certain environments by lactobacilli mainly occurs by their acid production, but can also be achieved by production of antagonistic substances (Jack *et al*, 1995; Hawes *et al*, 1996) or by competitive exclusion of pathogens (Hawrthorn and Reid, 1990).

Several authors (Doyle *et al*, 1990; Gibbons, 1996) have studied the strength of bacterial attachment. Piette and Idzíak (1992), reported that the cell charge and hydrophobicity influence the strength of adhesion. Studies of these non-specific interactions have led to the application of different methods such as bacterial adherence to plastics and glass, salting-out with increasing ammonium sulphate concentrations (Jonsson and Wadstrom, 1984), and extraction of bacteria from aqueous suspensions after treatment with hydrocarbons (Rosemberg *et al*, 1983; Sweet *et al*, 1987; Geertsema *et al*, 1993). Study of adhesion receptors also requires analysis of other properties such as haemagglutination (Andrew *et al*, 1995), prevalence of acid or basic bacterial surface characteristics (Pelletier *et al*, 1997), and coaggregation interactions and their mediators (Kolenbrander, 1991).

The aim of this work was to isolate and identify species of lactobacilli from different areas of the mouth (teeth, tongue, saliva and gum) of patients with optimal oral health, and to determine whether they possess adhesion-related properties.

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Materials and methods

Subjects

Oral health aspects previously described by Newbrun (1993), were considered for the selection of the subjects. The same dentist examined almost 170 individuals from Tucumán, Argentina, and determined their oral health status. Samples were only obtained from 44 subjects who met the conditions stated, and they were interviewed about their oral and nutritional habits for almost 30 min. The group consisted of 30 female and 14 male subjects aged between 9 and 28 years old with a high socio-economic level. Although only adults were scheduled to be included, no-one above the age of 28 with the stated conditions could be found. Similarly seven subjects with mixed dentition were also considered. Subjects were selected for having low DMFT (decayed, missing, filled permanent teeth), dmft (decayed, missing, filled deciduous teeth), DMFS (decayed, missing, filled permanent teeth surfaces) and dmfs (decayed, missing, filled deciduous teeth surfaces) indices; D, M, d and m components were 0 for all subjects. Observations with halogen light over mesial dental surfaces were performed and confirmed by X-rays only in doubtful situations. Plaque and Gingival indices and the number of sugar intakes per day were determined and salivary pH and secretion rates were registered.

None of the subjects had the habit of smoking, showed mucosal inflammatory lesions or were on orthodontic treatment. Neither had they taken in antibiotics or corticoids in the last 20 days. Subjects gave their consent and the Institutional Ethics Committee approved the experimental protocol.

Sample collection was performed in the morning after at least 2 h of fasting and hygiene, from four sites of the oral cavity: (a) buccal, lingual and occlusal surfaces of the left first mandibular molar and second mandibular premolar, either permanent or deciduous from mixed dentition subjects, (b) right half of the tongue, (c) 0.5 ml of saliva accumulated in the sublingual area during a controlled time, usually about 5 min, and (d) right occlusal mandibular gum. Teeth samples were taken with sterile Gracey No. 11/12 curettes, tongue and gum with sterile dental spatulas, and saliva with sterile syringes. Stress situations of the subjects previous to and during sampling were avoided.

Bacterial collection and conservation

Samples from teeth, tongue and gums of each subject were collected separately in 0.5 ml LBS (lactobacilli selected media) (Rogosa and Sharpe, 1963) broth; no broth was added to saliva samples. They were immediately put on ice and then transported to the laboratory and cultured on LBS agar plates under microaerophilic conditions (5% CO_2 water-jacketed Forma Scientific Incubator, Model 3185) up to 48 h at 37°C. Lactobacilli isolates were stored in milk-yeast extract (13% non-fat milk, 1% yeast extract) at −70°C.

Lactobacilli isolation and identification

The microorganisms were identified by Gram-staining and biochemical properties. Gram-positive, catalase-negative and indole and nitrate-reduction-negative bacilli were selected. Lactobacilli identification was performed by standard tests and by API 50 CH System (Biomerieux, France), according to Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986).

Lactobacilli surface characteristics

The microorganisms were subcultured in LAPTg (Raibaud *et al*, 1963) broth not more than three times prior to the studies. After 12 h of incubation at 37°C, strains were collected by centrifugation at 2000 rpm for 10 min and washed in saline solution. The following properties were analysed:

Hydrophobic partition Hexadecane, xylene and toluene were used following the microbial adhesion to hydrocarbons (MATH) method, first described by Rosemberg *et al* (1983), and modified by Geertsema *et al* (1993). Sweet *et al* (1987) included the use of xylene. The microorganisms suspended in saline solution were adjusted to an optical density (OD) between 0.4 and 0.6 at 600 nm. Then the solvent was added to aliquots of the microorganisms, mixing vigorously for 1 min. After the two layers had separated the lower aqueous phase was carefully removed and transferred to clean tubes. OD_{600} was determined, and the percentage of hydrophobicity was calculated from the OD_{600} differences (% hydrophobicity = OD_{before}–OD_{after}/OD_{before} × 100). Lactobacilli were classified in three groups: those with low hydrophobicity (0–35%), moderate hydrophobicity (36–70%), and high hydrophobicity (71–100%).

Basic and acidic surface characteristics The MATH method with chloroform and ethyl acetate as organic solvents was used according to Pelletier *et al* (1997). Since chloroform is a Lewis acid with avidity for substances that give off electrons, and ethyl acetate with basic characteristics reacts with Lewis acids, acidity or basicity of the bacterial cell surface can be determined with this method. Results were obtained from the calculation mentioned before (OD_{600}) and are expressed as the percentage of partition. Strains were classified into low (0–35%), moderately (36–70%) and highly (71–100%) charged microorganisms.

Salt aggregation test (SAT) (Jonsson and Wadstrom, 1984) This method is based on the aggregation that highly hydrophobic bacteria show in presence of ammonium sulphate at different concentrations. Centrifuged microorganisms were suspended in 0.02 M phosphate buffer saline (PBS) pH 6.8 up to a concentration of 10^9 CFU ml^{-1}. Aliquots of bacterial suspensions and different ammonium sulphate solutions (0.2–2 M) in PBS were mixed for 2 min on slides. Aggregation was determined by microscopic observation. Positive controls were treated with 4 M salt solution. Strains were classified into three groups: lactobacilli that aggregated with 0.2–2 M ammonium sulphate, those that did not aggregate with ammonium salt, and auto-aggregating lactobacilli.

Haemagglutination Microplate agglutination was performed (Andrew *et al*, 1995). Suspensions of 10^9 CFU ml^{-1} in saline solution were serially diluted in 50 μl round-bottom wells. They were mixed and incubated with ABO human red blood cells, washed with saline solution and adjusted

to 2%. After incubation for 60 min at 37°C and 24 h at 4°C, haemagglutination was visually determined. Titres are registered as the inverse of the highest bacterial dilution that produced agglutination.

Statistics
The Pearson coefficient was determined for the comparison between the organic solvents, and the Spearman coefficient for the associations between salt aggregation and autoaggregation. The ANOVA test was used for lactobacillus haemagglutination with ABO red cells.

Results

Subjects
From the 44 patients, seven had mixed dentition. Dental index values and means were determined for all subjects: DMFT: 2.39 ± 2.37 (D: 0, M: 0, F: 2.39 ± 2.37), dmft: 1.80 ± 1.09 (d: 0, m: 0, f: 1.80 ± 1.09), DMFS: 2.88 ± 2.78 (D: 0, M: 0, F: 2.88 ± 2.78), dmfs: 1.75 ± 1.50 (d: 0, m: 0, f: 1.75 ± 1.50), T + t: 2.00 ± 2.38, S + s: 3.75 ± 2.06, Plaque Index: 0.43 ± 0.31 and Gingival Index: 0.24 ± 0.21.

Only 36.3% of the subjects had less than four sugar intakes per day, and 15% brushed their teeth once a day, but almost 44% had dental care more than once a year and 33% used dental floss. All subjects had balanced diets with respect to carbohydrates, proteins, vitamins and lipids. Other nutritional habits registered included daily milk intake (58%) and daily water consumption (55%). Their salivary characteristics were considered normal: pH: 6.03 ± 0.41 and secretion rate: 0.33 ± 0.28 ml min^{-1} (Dawes, 1996).

Isolation and identification of lactobacilli
Lactobacilli were isolated from at least one area of the mouth of 23 out of the 44 subjects considered. Subjects did not show the same strain isolation pattern, and only in one of them lactobacilli were isolated from teeth, tongue, saliva and gum. This was a male subject of 23 years old with a DMFS: 0, who lived in Mendoza, Argentina, until recently.

Levels of 17.6% of the lactobacilli was isolated from teeth, 42.3% from the tongue, 28.2% from saliva and 11.8% from the gum. Table 1 shows the frequency of the lactobacilli isolates obtained from the four areas of study and their distribution in metabolic groups. Most strains were facultative heterofermenters (44.7%), 29.4% was obligate homofermenter and 25.9% was obligate heterofermenter. *L. salivarius* and *L. acidophilus* predominated in teeth, *L. fermentum* and *L. plantarum* in tongue, *L. fermentum* and *L. delbrueckii* sp *delbrueckii* in saliva and *L. rhamnosus* in the gum.

L. delbrueckii sp *delbrueckii* and *L. rhamnosus* were the only strains found in all of the four areas. Two species, *L. alimentarius* and *L. casei* sp *casei* were isolated only from saliva whereas *L. buchnerii* and *L. coryniformis* sp *L. coryniformis* were isolated only from the tongue.

Lactobacilli surface characteristics

Hydrophobic partition The individual data for all strains in relation to surface hydrophobicity evidenced in the presence of hexadecane, xylene and toluene are shown in Tables 2, 3 and 4. Values for the three solvents were high to moderate for almost 81% of the strains. Pearson correlation coefficient was statistically significant ($P < 0.05$) for the results of each of the three solvents used. A high number of lactobacilli from tongue, teeth and saliva showed high to moderate hydrophobicity, while strains with low hydrophobicity were mainly isolated from the tongue. *L. acidophilus* and *L. paracasei* sp *paracasei* isolates were mostly hydrophobic species, while *L. rhamnosus* showed high hydrophobicity for teeth and gum, and low hydrophobicity in tongue and saliva. A similar behaviour was observed for *L. plantarum* with high hydrophobicity for teeth and tongue and low hydrophobicity for saliva.

Acid and basic characteristics Tables 2, 3 and 4 show results of individual strains. Pearson coefficient did not indicate a linear relation ($P > 0.05$) between chloroform and ethyl acetate, and none between these solvents and hexadecane and toluene.

No prevalence of opposite surface charges was observed in the isolates from teeth and gum since results for both solvents were almost similar. Saliva and tongue had high basic-charged strains. An important number of isolates from the tongue also showed low solvents affinity that coincides with low hydrophobicity results. *L. delbrueckii* sp *delbrueckii* from teeth and gum, and *L. rhamnosus* from gum, characterised by high to moderate hydrophobic properties, showed high basic surface charge.

Salt aggregation and autoaggregation Individual results for SAT and autoaggregation are summarised in Tables 2, 3 and 4. Spearman coefficient was significant ($P < 0.05$) for SAT and autoaggregation results. However, these two properties did not necessarily coincide with the same strain, especially in teeth and tongue where some of them either salt aggregated or autoaggregated. Autoagglutination results correlated with those of hexadecane, chloroform and ethyl acetate. Almost 30% of the strains from teeth and tongue, 50% from saliva and 70% from gum aggregated.

Haemagglutination Only a few lactobacilli agglutinated ABO red blood cells, and there was no statistical difference ($P > 0.05$) (ANOVA) between titres for the three blood groups.

When all results were considered, correlation studies revealed significant linear relation between ABO agglutination, salt agglutination and autoagglutination, but not with the totality of the solvents (Tables 2, 3 and 4).

Discussion

Oral diseases seem to develop following a modification of the equilibrium of indigenous bacterial populations (Marsh, 1989). During this process, pathogenic bacteria have to adhere to the hard or soft tissues of the mouth in order to guarantee their colonisation. Bacterial adherence involves specific and non-specific mechanisms (Gibbons, 1996). The latter are related to electrostatic or hydrophobic interactions and have lower affinity than specific bindings.

The aim of our investigation is to study and elucidate

Table 1 Proportion of the lactobacilli species isolated from oral healthy mouth subjects

Species	*Metabolic group*	*Isolated strains (No. (%)*[a]			
		Teeth	*Tongue*	*Gum*	*Saliva*
L. acidophilus		3(20.0)(3.5)	1(2.8)(1.2)	–	1(4.2)(1.2)
L. delbrueckii sp *delbrueckii*	Obligated	1(6.7)(1.2)	3(8.3)(3.5)	1(10)(1.2)	4(16.7)(4.7)
L. delbrueckii sp *lactis*	Homofermenters	1(6.7)(1.2)	1(2.8)(1.2)	–	1(4.2)(1.2)
L. salivarius		4(26.7)(4.7)	2(5.5)(2.3)	–	2(8.3)(2.3)
L. agilis		–	1(2.8)(1.2)	2(20)(2.3)	1(4.2)(1.2)
L. alimentarius		–	–	–	2(8.3)(2.3)
L. bavaricus		–	–	1(10)(1.2)	–
L. brevis		1(6.7)(1.2)	1(2.8)(1.2)	–	–
L. casei sp casei	Facultative	–	–	–	1(4.2)(1.2)
L. rhamnosus	Heterofermenters	1(6.7)(1.2)	2(5.5)(2.3)	3(30)(3.5)	1(4.2)(1.2)
L. coryniformis sp *coryniformis*		–	1(2.8)(1.2)	–	–
L. maltaromicus		–	1(2.8)(1.2)	1(10)(1.2)	–
L. paracasei sp paracasei		2(13.3)(2.3)	1(2.8)(1.2)	–	2(8.3)(2.3)
L. plantarum		2(13.3)(2.3)	10(27.8)(11.8)	–	1(4.2)(1.2)
L. buchnerii	Obligated	–	1(2.8)(1.2)	–	–
L. fermentum	Heterofermenters	–	11(30.5)(12.9)	2(20)(2.3)	8(33.3)(9.4)
Total		**15(17.6)**	**36(42.3)**	**10(11.8)**	**24(28.2)**

[a] First number in brackets indicate the partial percentages referred to the area of the mouth, and second numbers in brackets indicate total percentages referred to the total number of lactobacilli isolated strains

Table 2 Adhesion-related properties of lactobacillus strains isolated from teeth

Isolated strains[a]	*Hydrophobicity (%)*			*Surface charge (%)*		*SAT (M)*[b]	*Auto-aggregation*[c]	*Haem-agglutination*[d]
	Hexadecane	*Xilene*	*Toluene*	*Chloroform*	*Ethyl acetate*			
L. acidophilus (41)	57	68	70	83	88	0	0	0
L. acidophilus (42)	70	80	73	88	95	0	0	0
L. acidophilus (45)	70	63	64	80	86	0	0	0
L. brevis (275)	52	27	11	22	16	0	0	0
L. rhamnosus (396)	75	87	87	90	92	0.2	0	0
L. delbrueckii sp *delbrueckii* (413)	90	82	74	94	24	0	+	A,B,O (1)
L. delbrueckii sp *lactis* (431)	69	88	70	82	88	0.2	0	0
L. parac. sp *paracasei* (341)	95	80	92	90	63	0.2	0	0
L. parac. sp *paracasei* (220)	90	92	88	92	77	0.2	0	0
L. plantarum (274)	84	90	87	37	45	0	0	0
L. plantarum (163)	65	58	56	86	86	0	0	0
L. salivarius (253)	65	73	81	92	92	0	0	0
L. salivarius (255)	47	50	31	78	91	0	0	0
L. salivarius (44)	80	80	80	85	93	0	0	0
L. salivarius (43)	44	40	38	57	60	0	0	0

[a] Numbers in brackets indicate the laboratory internal nomenclature
[b] SAT, salt aggregation test. Numbers indicate the minimal molar ammonium sulphate concentration that produced aggregation
[c] +: autoaggregation, 0: non-autoaggregation
[d] A, B, O: Blood groups lactobacilli aggregated with. Numbers in brackets indicate titres, that is, the inverse of the highest bacterial dilution that produce haemagglutination. 0: without haemaglutination

the role of lactobacilli in the ecology of oral environments focusing on their surface characteristics and their aggregation and co-aggregation capabilities. Therefore isolation and identification of these microorganisms from different groups of patients and from different areas of the oral cavity were performed (Ahumada *et al*, 1999). To date there are not many studies of the species of lactobacilli isolated from the mouth (Marsh, 1984). There are only few references to oral lactobacilli with probiotic-like properties. Meurman *et al* (1995), described a weak inhibitory substance from *Lactobacillus* GG against *Streptococcus sobrinus*. Recently, Straetemans *et al* (1998), found no differences in the number of lactobacilli between mutans streptococci-free and mutans streptococci-colonised children after the 'second window of infection'.

In this article the isolation of 85 microaerophilic *Lactobacillus* strains from teeth, tongue, saliva and gum from subjects with optimal nutritional and oral health is reported

Table 3 Adhesion-related properties of lactobacillus strains isolated from tongue

Isolated strains[a]	*Hydrophobicity (%)*			*Surface charge (%)*		*SAT (M)*[b]	*Auto-aggregation*[c]	*Haem-agglutination*[d]
	Hexadocane	*Xilene*	*Toluene*	*Chloroform*	*Ethyl acetate*			
L. acidophilus (40)	73	77	77	85	88	0	+	0
L. agilis (3901)	73	78	73	92	58	0.2	+	0
L. brevis (240)	79	70	80	38	2	0	+	0
L. buchnerii (25)	74	90	78	92	66	0	+	A, B, O(1)
L. rhamnosus (321)	0	0	0	4	52	0	0	0
L. rhamnosus (201)	4	0	4	0	37	0	0	0
L. coryn. sp coryniformis (254)	48	26	31	88	93	0	w	0
L. delbr. sp delbrueckii (441)	68	78	81	85	57	0.2	+	A, B, O(4)
L. delbr. sp delbrueckii (490)	15	8	6	7	4	0.2	+	A,B,0(4)
L. delbr. sp delbrueckii (3902)	85	78	89	89	46	0.2	+	A, B, O(1)
L. delbrueckii sp lactis (500)	25	33	19	45	30	0.5	0	0
L. fermentum (22)	71	84	78	87	71	2	0	0
L. fermentum (37)	82	81	94	81	88	0.5	0	0
L. fermentum (38)	66	77	82	87	24	0	0	0
L. fermentum (34)	68	79	79	98	85	0.5	0	0
L. fermentum (33)	79	80	92	97	81	0	+	0
L. fermentum (21)	70	90	94	83	72	2	w	0
L. fermentum (342)	21	13	18	16	10	0	0	0
L. fermentum (301)	67	63	57	39	18	0	0	0
L. fermentum (273)	52	70	60	55	60	0	0	0
L. fermentum (520)	20	45	35	45	0	0	0	0
L. fermentum (200)	70	80	72	76	88	0	+	0
L. maltaromicus (32)	64	71	67	60	34	0	0	0
L. parac. sp paracasei (28)	75	81	83	92	77	0	0	0
L. plantarum (272)	60	69	60	75	89	0	0	0
L. plantarum (381)	69	67	72	51	59	0	w	0
L. plantarum (382)	36	50	40	56	38	0	0	0
L. plantarum (384)	78	70	73	92	88	0	0	0
L. plantarum (393)	20	22	20	5	0	1	0	0
L. plantarum (392)	60	78	62	12	30	0.2	0	0
L. plantarum (4203)	8	13	13	18	47	0	0	0
L. plantarum (161)	72	77	75	85	92	0	+	0
L. plantarum (162)	40	62	75	80	87	0	0	0
L. plantarum (31)	14	28	24	64	90	0.2	0	0
L. salivarius (251)	15	36	30	49	94	0	+	0
L. salivarius (46)	86	62	66	73	89	0	0	0

[a] Numbers in brackets indicate the laboratory internal nomenclature
[b] SAT: Salt Aggregation Test. Numbers indicate the minimal molar ammonium sulphate concentration that produced aggregation
[c] +: autoaggregation, w: weakly autoaggregation, 0: non-autoaggregation
[d] A, B, O: Blood groups lactobacilli aggregated with. Numbers in brackets indicate titres, that is, the inverse of the highest bacterial dilution that produce haemagglutination. 0: without haemaglutination

with the purpose of screening bacterial surface characteristics and predicting adhesive properties. This is the first step in the selection of species with probiotic properties *L. fermentum*, *L. plantarum*, *L. delbrueckii* sp *delbrueckii*, *L. salivarius* and *L. rhamnosus* were the most numerous isolates. *L. delbrueckii* sp *delbrueckii* strains were selected for further studies because of their frequency and properties, while *L. rhamnosus* was chosen because of its different behaviour in tongue and saliva with respect to teeth and gum.

Adhesive lactobacilli properties have been described previously for other environments (Harty and Knox, 1991; Garriga *et al*, 1998). The hydrophobic nature of the intestinal mucous layer suggests that a hydrophobic bacterial surface is necessary for non-specific interaction with mucin, the glycoproteic intestinal layer (Malagelada, 1998). Mucin is also present in saliva, and should therefore be included in further adhesion studies. Most of the strains isolated had high to moderate hydrophobicity in presence of any of the solvents assayed (hexadecane, xylene and toluene). These results do not coincide with those obtained for strains isolated from non-healthy mouths, where only 20% was highly hydrophobic and 50% showed low hydrophobicity (Ahumada *et al*, 1999).

Lactobacilli from healthy mouths are highly charged notwithstanding their lack of predominant surface charge. A negative correlation between chloroform and ethyl acetate as reported by Pelletier *et al* (1997), was not observed, suggesting that lactobacilli from oral environments possess different surface characteristics.

Haemagglutination property was tested in order to analyse the presence of lectins on the bacterial surface (Piette and Idziak, 1992). This method is based on the similarity in structure that could exist between epithelial mammalian cell receptors (host) and red blood cells. Mukai *et al* (1998) reported that a β-galactosyl residue at the non-reducing ter-

Table 4 Adhesion-related properties of lactobacillus strains isolated from saliva and gum

	Hydrophobicity (%)			*Surface charge (%)*		*SAT (M)*[b]	*Auto-aggregation*[c]	*Haem-agglutination*[d]
	Hexadecane	*Xylene*	*Toluene*	*Chloroform*	*Ethyl acetate*			
Isolated strains from saliva[a]								
L. acidophilus (465)	77	84	78	82	63	0	0	0
L. agilis (160)	59	76	59	83	27	2	0	0
L. alimentarius (414)	35	37	53	93	83	0.2	+	0
L. alimentarius (4201)	59	69	75	91	69	0.2	+	A,B,O(1)
L. casei ssp.casei (291)	64	70	70	88	70	0	0	0
L. rhamnosus (302)	12	2	7	44	14	0	+	0
L. delbr sp delbrueckii (461)	76	89	81	90	72	0.2	+	A,B,O(4)
L. delbr sp delbrueckii (462)	85	96	96	87	60	0.2	+	A,B,O(4)
L. delbr sp delbrueckii (463)	90	92	93	89	75	0.2	+	A,B,O(4)
L. delb sp delbrueckii (4202)	55	52	47	85	82	0	+	0
L. delbruecki sp lactis (464)	83	96	90	88	87	0.2	+	A,B,O(4)
L. fermentum (29)	63	52	52	64	52	0	0	0
L. fermentum (36)	76	91	81	98	76	1.5	0	0
L. fermentum (23)	72	78	80	92	85	0.2	0	A,B,O(2)
L. fermentum (340)	75	64	80	72	75	0.2	w	0
L. fermentum (391)	50	38	22	83	60	0.2	+	0
L. fermentum (522)	40	67	57	80	93	0	0	0
L. fermentum (521)	73	67	71	10	13	0	+	0
L. fermentum (132)	75	62	56	3	10	0	+	0
L. paracasei sp paracasei (35)	76	86	98	79	36	0	0	0
L. paracasei sp paracasei (383)	38	69	48	60	38	0	0	0
L. plantarum (202)	10	0	15	4	0	0	0	0
L. salivarius (250)	42	65	50	90	90	0	0	0
L. salivarius (510)	28	35	38	40	0	0	0	0
Isolated strains from gum[a]								
L. agilis (320)	46	56	62	86	86	0	0	0
L. agilis (480)	40	66	20	50	65	0	0	0
L. bavaricus (397)	67	72	65	96	85	0.2	+	A,B,O(4)
L. rhamnosus (26)	76	78	81	83	23	0.2	+	A,B,O(1)
L. rhamnosus (398)	83	87	85	92	77	0.2	+	0
L. rhamnosus (399)	82	93	92	65	75	0.2	+	0
L. delbr sp delbrueckii (411)	47	58	60	92	17	0	0	0
L. fermentum (24)	85	90	88	70	89	0.2	0	0
L. fermentum (27)	40	35	39	65	85	0.2	0	0
L. maltaromicus (412)	90	85	88	92	83	0.2	+	A,B,O(2)

[a] Numbers in brackets indicate the laboratory internal nomenclature
[b] SAT: Salt Aggregation Test. Numbers indicate the minimal molar ammonium sulphate concentration that produced aggregation
[c] +: autoaggregation, w: weakly autoaggregation, 0: non-autoaggregation
[d] A, B, O: Blood groups lactobacilli aggregated with. Numbers in brackets indicate titres, that is, the inverse of the highest bacterial dilution that produce haemagglutination. 0: without haemagglutination

minal of bacterial surface glycoconjugates would be responsible for adhesion of *L. reuteri* to the intestinal mucosa, behaviour that is strain-specific. The number of our strains that haemagglutinated, even those from the tongue, was low, and no statistically significant differences were obtained between human red blood cell groups. It could be suggested that interactions similar to those produced between oral lactobacilli and blood cells would not be the most important ones, at least not under the tested conditions. These results are in agreement with previous works, since only Andrew *et al* (1995), reported haemagglutination property for lactobacilli of vaginal origin.

Lactobacilli autoaggregate and aggregate in the presence of ammonium sulphate, especially those from the gum. Autoagglutination or co-aggregation is a very important property since co-aggregation with undesirable bacteria could regulate the oral ecology by swallowing. However, this property could also contribute to a symbiotic process between lactobacilli and cariogenic adhering bacteria. Further studies are needed to elucidate the importance of this behaviour with respect to retention or elimination of bacteria from the oral cavity.

Recently, Kaiserlian (1998) expressed that the mucosa of the mouth does not show immunological tolerance in contrast to the intestinal mucous membrane. Lactobacilli influence on the immune system should be considered in relation to their probable probiotic use where the oral cavity is involved directly.

This study proves that the bacterial cell surface of lactic acid bacteria contains different structures. The surface charge could be involved in their adhesion to hard or soft tissues of the mouth, or in attachment to other bacteria. Lactobacilli could be associated with caries development, or they could promote certain other functions related to the

regulation of the ecology and the presence of pathogenic bacteria in the oral cavity.

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References

Ahumada MC, Colloca ME, López ME *et al* (1999). Characterisation of lactobacilli isolated from tongue and gum. *Anaerobe* **5**: 000–000.

Andrew A, Stapleton A, Fennell S *et al* (1995). Haemagglutination, adherence and surface properties of vaginal lactobacillus species. *J Inf Dis* **171**: 1237–1245.

Dawes C (1996). Factors influencing salivary flow rate and composition. In: Edgar WM, O'Mullane DM, eds. *Saliva and Oral Health*. British Dental Association Publications: London, pp 27–41.

Doyle RJ, Rosenberg M, Drake D (1990). Hydrophobicity of oral bacteria In: Doyle RJ, Rosenberg M, eds. *Microbial Cell Surface Hydrophobicity*. Am Soc Microbiol Publications: Washington DC, pp 387–419.

Garriga M, Pascual M, Monfort JM *et al* (1998). Selection of lactobacilli for chicken probiotic adjuncts. *J Appl Microbiol* **84**: 125–132.

Geertsema GI, Van der Mei HC, Busscher HJ (1993). Microbial cell surface hydrophobicity. The involvement of electrostatic interactions in microbial adhesion to hydrocarbons (MATH). *J Microbiol Methods* **18**: 61–68.

Gibbons RJ (1996). Role of adhesion in microbial colonisation of host tissues: a contribution of oral microbiology. *J Dent Res* **75**: 866–870.

Harty DWS, Knox KW (1991). An *in vitro* study of adhesion of various Lactobacillus species. *Microb Ecol in Health and Dis* **4**: 19–28.

Hawes SE, Hillier SL, Benedetti J *et al* (1996). Hydrogen-peroxide producing lactobacilli and acquisition of vaginal infections. *J Infect Dis* **174**: 1058–1063.

Hawrthorn L, Reid G (1990). Exclusion of uropathogen adhesion to polymer surfaces by *Lactobacillus acidophilus*. *J Biomed Mat Res* **24**: 39–46.

Jack RW, Tagg JR, Ray B (1995). Bacteriocins of Gram-positive bacteria. *Microbiol Rev* **59**: 171–200.

Jonsson P, Wadstrom T (1984). Cell-surface hydrophobicity of *Staphylococcus aureus* measured by the Salt Aggregation Test (SAT). *Curr Microbiol* **10**: 203–210.

Kaiserlian D (1998). Immunity and tolerance at leuco-epithelial mucosal interfaces. Fermented Food, Fermentation and Intestinal Flora. Symposium Danone. 'Immunity and Probiotics', Denmark.

Kandler O, Weiss N (1986). Regular, non-spore forming Gram positive rods. In: Sneath PA, ed. *Bergey Manual of Systematic Bacteriology*. Williams and Wilkins Co: Baltimore, pp 1209–1235.

Kolenbrander PE (1991). Coaggregation: adherence in the human oral microbial ecosystem. In: Dworkin M, ed. *Microbial Cell–Cell Interactions*. American Society of Microbiology Publishers: Washington DC, pp 303–329.

Malagelada JR (1998). Non immunological models of protection. Fermented Food. Fermentation and Intestinal Flora. Symposium Danone. 'Immunity and Probiotics', Denmark.

Marsh P (1984). The normal oral flora. In: Cole JA, Knowles CJ, Schlessinger D, eds. *Oral Microbiology*. Surrey, pp 11–24.

Marsh PD (1989). Host defences and microbial homeostasis: role of microbial interactions. *J Dent Res* **68**: 1567–1575.

Meurman JH, Antila H, Korhonen A *et al* (1995). Effect of *Lactobacillus rhamnosus* strain GG (ATCC 53103) on the growth of *Streptococcus sobrinus in vitro*. *Eur J Oral Sci* **103**: 253–258.

Mukai T, Kaneko S, Ohori H (1998). Haemagglutination and glycolipid-binding activities of *Lactobacillus reuteri*. *Lett Appl Microbiol* **27**: 130–134.

Nader-Macías ME, Silva-Ruiz C, López-Bocanera ME *et al* (1996). Behaviour of lactobacilli on prevention and therapeutic effects on urinary tract infections (UTI) in mice. *Anaerobe* **2**: 85–93.

Newbrun E (1993). Problems in caries diagnosis. *Int Dent J* **43**: 133–142.

Pelletier C, Bouley C, Cayuela C *et al* (1997). Cell surface characteristics of *L. casei ss casei, L. paracasei ss paracasei* and *L. rhamnosus* strains. *Appl Envirom Microbiol* **3**: 1725–1731.

Piette JPG, Idziak ES (1992). A model study of factors involved in adhesion of *Pseudomonas fluorescens*. *Appl Envirom Microbiol* **58**: 2783–2791.

Prakobphol A, Burdsal CA, Fisher SJ (1995). Quantifying the strength of bacterial adhesive interactions with salivary glycoproteins. *J Dent Res* **74**: 1212–1218.

Raibaud P, Galpin JV, Ducluzeau R *et al* (1963). Le genre Lactobacillus dans le tube digestif du rat. II Caracters de souches heterofermentaires isolees de rats. 'Holo' et 'Gnotoxeniques' Annales de Microbiologie. *Annales de L'Institutu Pasteur* **124A**: 2223–2235.

Redondo-López V, Cook RL, Sobel JD (1990). Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis* **12**: 856–872.

Rogosa M, Sharpe E (1963). Species differentiation of human vaginal lactobacilli. *J Gen Microbiol* **23**: 197–201.

Rosemberg M, Judes H, Weiss, E (1983). Cell surface hydrophobicity of dental plaque microorganisms *in situ*. *Infect Immun* **42**: 831–834.

Straetemans MME, van Loveren C, de Soet JJ *et al* (1998). Colonisation with mutans streptococci and lactobacilli and the caries experience of children after the age of five. *J Dent Res* **77**: 1851–1855.

Sweet S, Wallace T, Samaranayake L (1987). Determination of the cell surface hydrophobicity of oral bacteria using a modified hydrocarbon adherence method. *FEMS Microbiol Lett* **48**: 159–163.