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Surface characteristics of lactobacilli isolated from human vagina

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In the present paper, the taxonomic classification of 134 lactobacilli isolates from vaginal samples of 200 women of Tucumán, Argentina, is reported. They were clustered in three metabolic groups of the genus *Lactobacillus*, most belonging to the obligately homofermentative group (56%), mainly represented by *Lactobacillus delbrueckii* subsp. *delbrueckii* and *L. acidophilus.* In the facultatively heterofermentative group (24%), the dominant species were *L. paracasei* subsp. *paracasei* and *L. agilis*, and in the obligately heterofermentative group (20%), *L. brevis* was the dominant species. All strains were studied for surface characteristics and adhesion-predicting properties. A correlation between the methods employed for hydrophobicity testing of the different isolates (Microbial Adhesion to Hydrocarbons and Salt Aggregation Test) is reported. Most strains were highly hydrophobic. Their hemagglutination capability with human erythrocytes was also tested, which was positive only for a few strains. Some isolates were self-aggregating. From our results, strains that shared the properties assayed were selected for further testing of some other desirable characteristics, such as antagonistic substance production, adhesion to biological substrates, and appropriate technological properties, to suggest the elaboration of a probiotic for the vaginal tract.

Key Words——adhesion; hydrophobicity; probiotics; surface characteristics; vaginal lactobacilli

Lactobacilli are the dominant bacteria of a healthy human vagina (Redondo-López et al., 1990) and are routinely found as part of a well-defined “normal” or “indigenous” microflora in the female reproductive tract, first described by Rogosa and Sharpe (1963). More recently, Lachlack et al. (1996) reported the *Lactobacillus acidophilus* complex, isolated from the vaginal flora in 150 human samples. Furthermore, Reid et al. (1996) identified *Lactobacillus* species from the vagina of 100 healthy women. There is no consensus on which *Lactobacillus* species are the main ones in the human vagina, but there is agreement on their number, which is about 10^7–10^8 CFU/g of fluid (Redondo-López et al., 1990).

The vagina has been increasingly recognized as an ecosystem of which the natural flora helps to avoid the invasion of pathogens. The function of lactobacilli is to maintain an environment that restricts the growth of pathogenic microorganisms (Redondo-López et al., 1990). The female reproductive tract is susceptible to a variety of viral and bacterial infections, including those caused by human papilloma and human herpes simplex viruses, *Chlamydia trachomatis*, GAM complex, *Neisseria gonorrhoeae*, and protozoa such as *Trichomonas vaginalis* (Rush et al., 1995). Many studies have shown a correlation between the disruption of the normal genital microflora, especially of the *Lactobacillus* species, and an increase in genital and bladder infections (Redondo-López et al., 1990). These observations have led to scientific research on the strains and properties of vaginal lactobacilli, which might be responsible for the maintenance of a noninfectious ecosystem. It has been suggested that these properties and some antagonistic mechanisms may include competitive interference in pathogen adhesion (Chan et al., 1985), capability to aggregate with other bacteria (Boris et al., 1998), and production of bacteriocin-like (Jack et al., 1995) or antibiotic-like substances, hydrogen peroxide (Klebanoff et al., 1991), or biosurfactants (Velraeds et al., 1996). Competition

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of nutrients (Freter et al., 1983) and stimulation of the immune system (Witkin, 1993) may also affect the balance of the integrated microbial community.

In this paper, the vaginal flora of women under gynecological control is studied to isolate and identify lactobacilli. Different techniques that can be applied to the prediction of adhesion characteristics of lactobacilli were performed: microbial adhesion to hydrocarbons, Salt Aggregation Test (SAT), and hemagglutination (HA). Correlation among the results obtained with these different techniques was evaluated. Strains showing optimal adhesion properties were selected for further testing of the desirable characteristics of a probiotic, which would allow us to design a product for local application to the vaginal tract.

Materials and Methods

Vaginal samples. Samples were obtained from 200 women from 19 to 45 years old under gynecological control. Consent was obtained from all participants, and the Institutional Ethics Committee approved the protocol. The samples were taken aseptically with a cotton swab, collected in LBS (*Lactobacillus* Selective Medium) (Rogosa and Sharpe, 1963) broth, and taken to the laboratory where they were transferred to different culture media.

Isolation and identification of microorganisms. Lactobacilli were first enriched in LBS, then grown on LBS agar and LAPTg agar (Raibaud et al., 1963). Plates were incubated in a microaerophyllic environment at 37°C for up to 48 h. The microorganisms selected were Gram-positive, catalase-negative, indole-negative, and nitrate reduction-negative bacilli. Species of *Lactobacillus* were identified by biochemical profiles, sugar fermentation patterns, and the API CH 50 system (BioMérieux Vitec, Inc., Lyon, France), according to Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986). They were stored in milk-yeast extract (13% nonfat milk, 1% yeast extract) at −70°C.

Surface characteristics. HA ability: The frozen microorganisms were subcultured in LAPTg broth three times before the performance of all further studies. After incubation at 37°C for 12 h, they were collected by centrifugation and washed with saline. Suspensions of 10^9 CFU/ml were prepared and serially diluted (1/2, 1/4, 1/8) in round-bottom microplates. Red blood cells (RBC) (human groups A, B, and O) were washed with the same solution, adjusted to 2% (vol/vol), and aliquots were added to the wells. Bacteria and RBC were mixed together and incubated at 37°C for 30 min and at 4°C for 6 h. HA was visually determined. The results were expressed as the inverse of the highest dilution of bacteria producing agglutination.

Hydrophobic partition: For the assessment of the degree of hydrophobicity, Microbial Adhesion to Hydrocarbons (MATH) method was employed. The technique used was first described by Rosenberg et al. (1983) and modified by Geertsema et al. (1993). Three different hydrophobic solvents were employed: hexadecane, xylene, and toluene. Bacterial pellets were washed and resuspended in saline to obtain an optical density (OD_{600}) from 0.4 to 0.6. The solvent was then added to each cellular suspension and the mixture vortexed vigorously for 1 min. They were kept still to allow the immiscible solvent and aqueous phase to separate. The lower aqueous layer was carefully removed by using Pasteur pipettes, and transferred to clean tubes. Absorbance was measured as before. The percent of hydrophobicity was obtained from the following calculation:

$$\%\ \text{hydrophobicity} = \frac{OD_{before} - OD_{after}}{OD_{before}} \times 100$$

Chloroform and ethyl acetate partition: By the use of the same methodology as MATH, but with chloroform and ethyl acetate, the basic and acid characteristics of the cell surface were determined (Pelletier et al., 1997). Chloroform is a Lewis acid, electron acceptor, with avidity for these substances able to give electrons (Lewis bases). Similarly, ethyl acetate, with basic characteristics, is avid for acid substances. The results of this test are expressed as the percentage of strains with avidity for acid and basic solvents, calculated with the same formula as used before.

SAT: SAT is another method described for determining the hydrophobic characteristics of the bacterial surface (Lindahl et al., 1981). Highly hydrophobic bacteria aggregate according to characteristic patterns in the presence of ammonium sulfate. Cells cultured in LAPTg were collected by centrifugation, washed, and resuspended in phosphate buffer saline (PBS) (0.02 M pH 6.8) to get a bacterial concentration of 10^9 CFU/ml. Ammonium sulfate in PBS was prepared at different concentrations (2 to 0.2 M). Aliquots of bacterial suspensions and salt solutions were mixed for 2 min on slides. Agglutination capability was determined by observation under a microscope. The strains were clustered in three different groups: strains able to aggregate with ammonium sulfate, strains without aggregative properties, and strains able to autoaggregate (aggregation in the presence or absence of salt).

Statistical analysis. The multivariate techniques used in this study include cluster and factor analysis. Cluster analysis finds groups of similar observations based on a set of variables. The clustering method forms a predetermined number of groups of disjointed clusters in such a way that the distances between

them are maximal. The Euclidean distance between their centers was used for the group formation (Chatfield and Collins, 1992). In this paper, the hexadecane, xylene, and toluene variables formed three groups or clusters, and four were obtained for the HA degree with different RBC.

Spearman correlations were calculated between ordinal categorical variables, and the Pearson correlation was determined for continuous measurements.

Results

Lactobacillus species and groups

The microaerophilic lactobacilli recovered from 200 vaginal samples belonged to 134 different isolates. Anaerobic lactobacilli and accompanying flora were not determined in this study. Lactobacilli were identified on the basis of Gram-staining morphology, biochemical profiles, and API CH50 system, which allowed their classification in the three metabolic groups of the genus *Lactobacillus.* Most strains belonged to the obligately homofermentative group (56%), with *Lactobacillus delbrueckii* subsp. *delbrueckii* and *L. acidophilus* as the most frequent species. In the facultatively heterofermentative group (24%), the dominant species were *L. paracasei* subsp. *paracasei* and *L. agilis*; *L. brevis* was predominant in the obligately heterofermentative group (20%). The relative percentages of the species of each metabolic group are represented in Fig. 1a, b and c.

Surface characteristics

HA ability

The HA characteristics of lactobacilli tested with different groups of RBC are shown in Table 1. Most strains showed no HA ability: 63% of the strains with group A RBC, 64% with group B, and 47% with group O. The highest percentage of strains with high HA corresponded to obligately homofermentative lactobacilli with O RBC (17% of the total of isolates).

The relationship between the number of different *Lactobacillus* strains, different bacterial dilutions, and their ability to agglutinate with A, B, and O RBC allowed us to divide the strains into four clusters (Fig. 2a): cluster 1, strains with high HA degree (HA at high dilution); cluster 2, strains with medium degree (at dilutions from 1/2 to 1/4); cluster 3 showing HA only with group O; and cluster 4 with strains having no HA ability. A low number of strains hemagglutinated simultaneously with the three different blood groups. The correlation between HA with A and B, A and O, or B and O RBC is reflected in Fig. 2b. There is more similarity in the behaviour of the strains with A and B groups than with the others, as shown also in Fig. 2a. To determine if this behaviour was associated with a particular metabolic group, Spearman correlation coefficient was determined. It was observed that there exists a strong association between high HA ability with A and high HA ability with B RBC only in the obligately homofermentative and the facultatively heterofermentative groups (r: 0.93 and r: 0.89 respectively). Other correlation coefficients were not relevant.

Hydrophobic characteristics: MATH tested by partition in hexadecane, xylene, and toluene

To define hydrophobic characteristics, the strains were divided into 3 categories: strains with high (71–100%), medium (36–70%), and low (0–35%) hydrophobicity. The percentage of strains in the different categories is shown in Table 2. Most *Lactobacillus* isolates in the three metabolic groups show a high degree of hydrophobicity with the three solvents tested. The highest proportion of hydrophobic strains is in the homofermenters. By using the cluster formation technique with the variables hexadecane, xylene, and toluene, we also got three different groups of strains, which coincides with our first division in high, medium, and low hydrophobicity, as shown in Fig. 3a. While histograms were performed of the distribution frequency of hydrophobicity with the three solvents used (Fig. 3b), most strains had a degree of hydrophobicity higher than 70%.

Figure 3c shows the linear correlation between the different solvents used. This behavior is similar in the three metabolic groups. The figure also shows the Pearson correlation coefficients obtained.

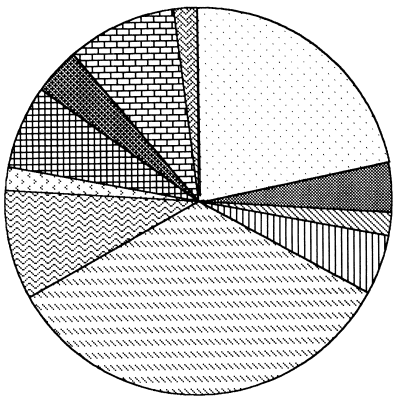
SAT

Forty-five percent of the isolates aggregated in the presence of ammonium sulfate (concentrations from 0.2 to 2 M), showing “salt aggregation” characteristics. Some strains produced self-aggregation (31% of the total strains), i.e., they aggregated in the presence or absence of salt. Salt aggregation is not associated with any particular metabolic group or species; instead, it is a property associated with certain strains.

No positive or negative correlation existed between the degree of SAT (considering the different concentrations) and the degree of hydrophobicity tested by MATH with hexadecane, xylene, or toluene. However, strains with high SAT showed a tendency to have high MATH. Those with low SAT had a wide range of MATH results. On the other hand, a high MATH did not guarantee a high SAT (data not shown).

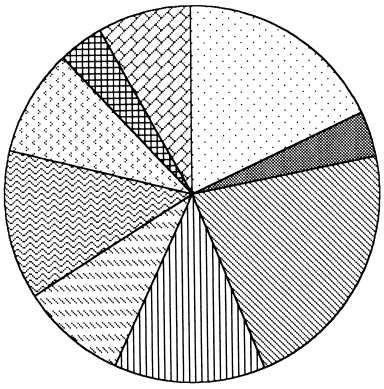
Chloroform and ethyl acetate

The percentages obtained by the MATH method with chloroform and ethyl acetate, acid, and basic solvents, respectively, are summarized in Table 3. Most isolates showed low affinity for both organic solvents. Pelletier et al. (1997) found that strains tested with chloroform and ethyl acetate presented an important difference between the results obtained with both sol-



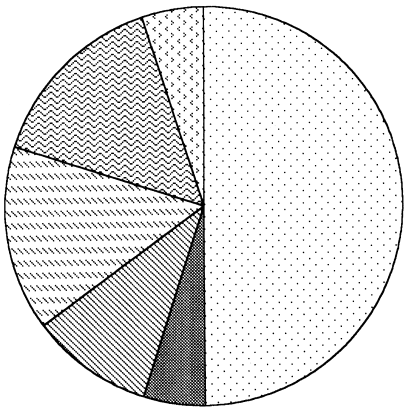
a

- *L. acidophilus*
- *L. amilophilus*
- *L. crispatus*
- *L. delbrueckii* subsp. *bulgaricus*
- *L. delbrueckii* subsp. *delbrueckii*
- *L. delbrueckii* subsp. *lactis*
- *L. fructosus*
- *L. gasseri*
- *L. helveticus*
- *L. jensenii*
- *L. salivarius*



b

- *L. agilis*
- *L. casei* subsp. *casei*
- *L. paracasei* subsp. *paracasei*
- *L. rhamnosus*
- *L. paracasei* subsp. *tolerans*
- *L. coryniformis* subsp. *coryniformis*
- *L. homohiochii*
- *L. plantarum*
- *L. sakei*



c

- *L. brevis*
- *L. buchnerii*
- *Carnobacterium divergens*
- *L. fermentum*
- *L. reuteri*
- *L. vaccinostercus*

Fig. 1. Frequency of different species of lactobacilli isolated from vaginal samples.
a: Obligately homofermentative group, b: Facultatively heterofermentative group, and c: Obligately heterofermentative group. The isolation and identification methods are described in the text.

Table 1. Percentage of lactobacilli isolates producing HA.

Inverse dilution	A RBC				B RBC				O RBC			
	OHO	FHE	OHE	Total	OHO	FHE	OHE	Total	OHO	FHE	OHE	Total
0	31	17	15	63	32	17	15	64	21	15	1	47
1	6	1	3	10	10	1	3	15	7	2	3	13
2	5	4	1	10	2	4	1	7	3	2	2	7
4	7	0	0	7	5	0	0	5	8	2	0	10
8	6	2	1	9	6	2	1	9	17	3	3	23

OHO: obligately homofermentative group. FHE: facultatively heterofermentative group. OHE: obligately heterofermentative group. 0: no HA. 1 to 8: inverse of the highest dilution producing HA. The method is described in the text.

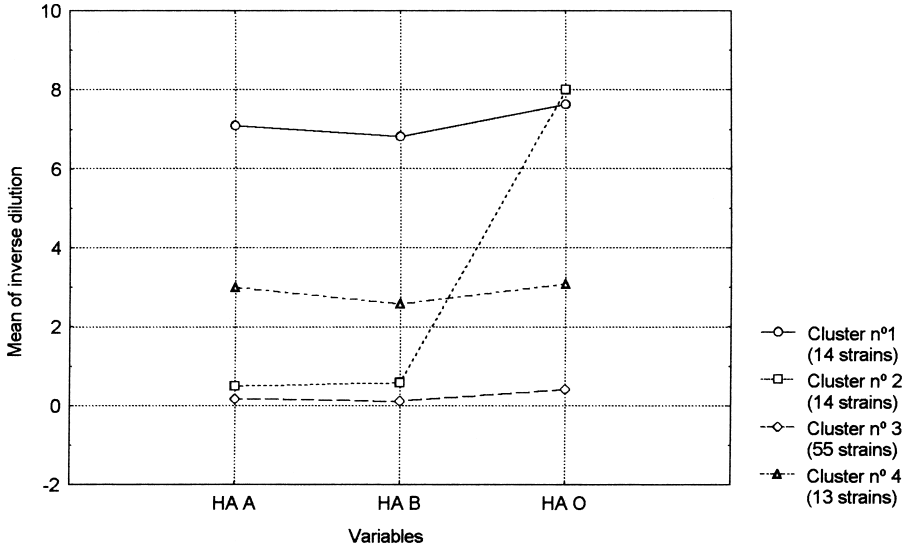


Fig. 2a. Cluster analysis of the HA test.
The plot of HA means for each cluster of *Lactobacillus* strains. The technique is described in the text.

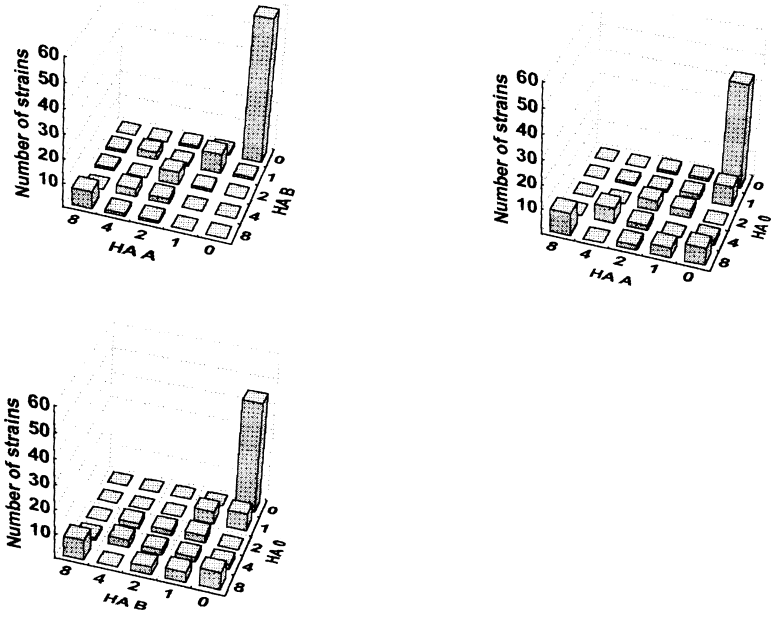


Fig. 2b. Relationships (expressed as percentages of strains) of HA with A and B, A and O, and B and O groups of RBC.

vents. In our results, the percentage of strains with a difference higher than 50% was only 20%.

Selection of lactobacilli

After the study of adhesion-predicting properties of lactobacilli by using different techniques and the comparison of methods employed, the strains that shared at least two desired characteristics were selected. The properties taken into account were: a) high hydrophobicity; b) positive HA with A, B, and O; and c) positive salt aggregation or self-aggregation. The selected *Lactobacillus* strains are shown in Table 4. These strains are being further studied to select those with optimal probiotic characteristics.

Discussion

Lactobacilli have been increasingly recognized as the predominant microorganisms of the healthy vaginal tract, helping to prevent pathogen onset (Redondo-López et al., 1990). On the other hand, a new concept has been introduced in the microbiology and ecology areas, with respect to the reestablishment of the indigenous flora, as based on the first proclamation of Metchnikoff (1907). Following this principle is an increasing tendency to the establishment of a preventive medicine, together with a higher use and consumption of probiotics. The term "probiotics" was redefined by Havenaar et al. (1992) as "a viable mono or mixed culture of microorganisms which, applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora." Freter

Table 2. Percentage of lactobacilli isolates with different degree of hydrophobicity tested by MATH.

Level	Hexadecane				Xylene				Toluene			
	OHO	FHE	OHE	Total	OHO	FHE	OHE	Total	OHO	FHE	OHE	Total
Low	14	23	26		18	31	26		23	35	26	
Total	8	6	5	19	10	8	5	23	13	9	5	26
Medium	21	31	32		32	23	26		23	19	32	
Total	12	8	6	25	18	6	5	28	13	5	6	24
High	65	46	42		51	46	47		54	46	42	
Total	36	12	8	56	28	12	9	49	30	12	8	50
All Grps	100	100	100	100	100	100	100	100	100	100	100	100
Total	56	25	19	100	56	25	19	100	56	25	19	100

Partition of hexadecane, xylene, and toluene determined three levels of hydrophobicity: Low: 0–35%, Medium: 36–70%, High: 71–100%. White rows: percentage refers to each metabolic group. Grey rows: percentage refers to the total of isolates. The method is described in the text.

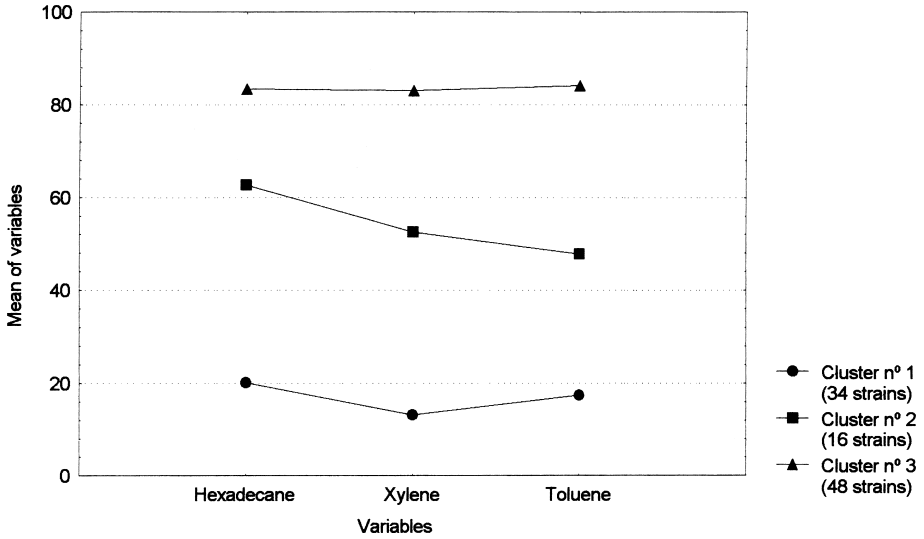


Fig. 3a. Cluster analysis of the MATH test.
The plot of hydrophobicity means for each cluster of lactobacilli strains.

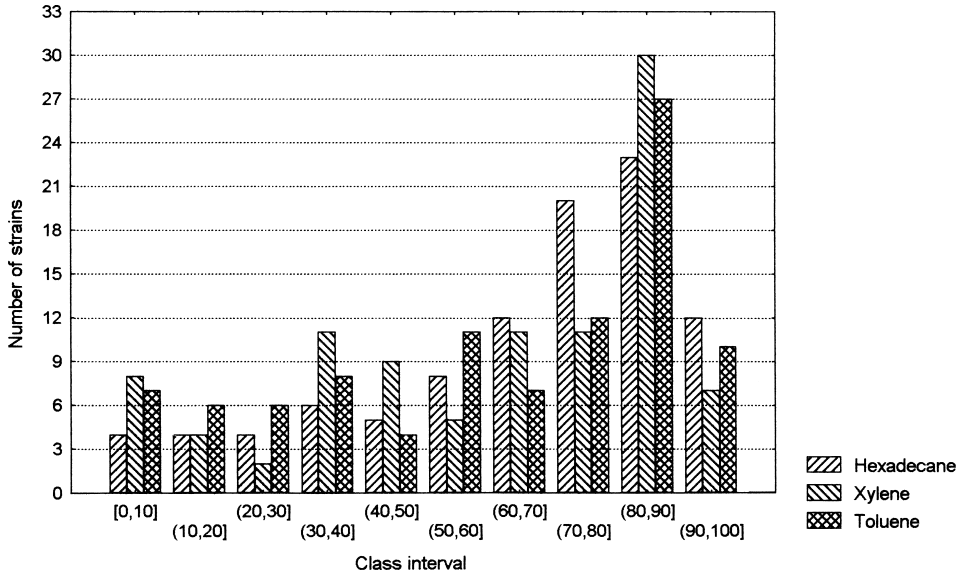


Fig. 3b. Frequency distribution of hydrophobicity of *Lactobacillus* strains tested with hexadecane, xylene, and toluene by the MATH method.

For the degree of hydrophobicity, class intervals of 10% were chosen.

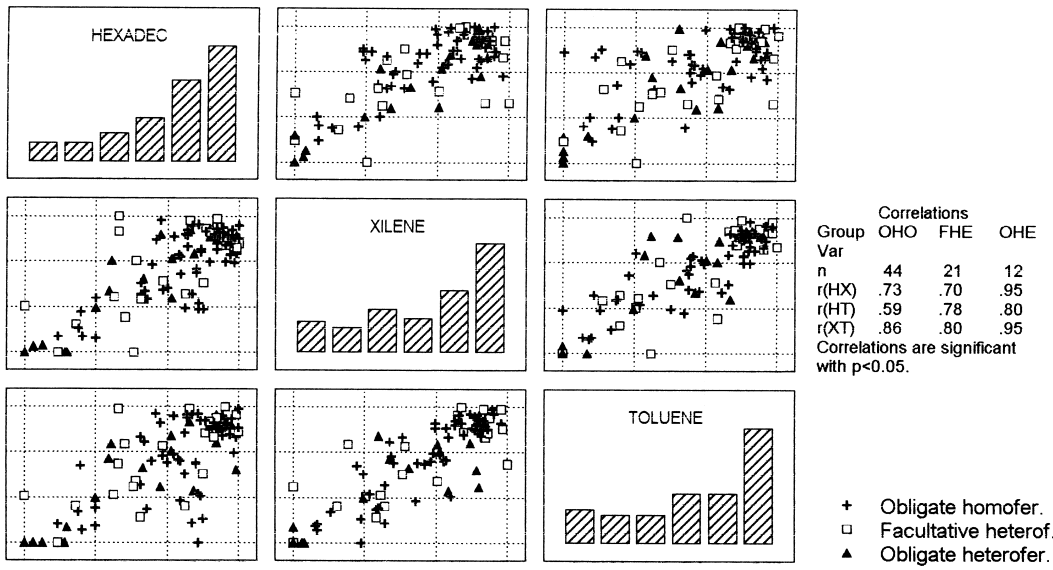


Fig. 3c. Matrix plot showing the relationship between the degree of hydrophobicity tested by MATH with hexadecane, xylene, and toluene.

r (HX): hexadecane and xylene, *r* (HT): hexadecane and toluene, *r* (XT): xylene and toluene. Pearson coefficients obtained are also indicated.

and Nader-Macías (1995) suggested another modification of this probiotics definition considering not only viable microorganisms, but also their metabolic products or cell compounds, administered to improve the microflora of the host and also to stimulate the immune system.

McGroarty (1993) and Parent et al. (1996) outlined some of the characteristics that probiotics for the urogenital tract should have, most of them referring to technological properties. Some experiments and assays reported on the prevention of urinary tract infections (Baerheim et al., 1994; Reid et al., 1992), and others referred to the vaginal tract. Hilton et al. (1995) mentioned the use of *Lactobacillus* GG isolated from the gastrointestinal tract, topically applied to the treatment of recurrent vaginitis. Hawes et al. (1996) reported that H_2O_2-producing lactobacilli protect against the acquisition of bacterial vaginosis, but not against vulvovaginal candidiasis or vaginal trichomoniasis. In this area, there are still many questions referring to

the mechanisms involved in the probiotic effects and in the prevention of infections. One objective of our group is the use of lactobacilli as a preventive measure in the urogenital tract; we began studying the protective effect of *L. fermentum* on this tract by using mice as experimental models (Nader-Macías et al., 1992, 1996; Silva-Ruíz et al., 1993, 1996). In the present paper, we report the isolation of 134 strains belonging to the three metabolic groups of lactobacilli. A combination of morphological and biochemical studies, complementary to the API CH50 test, were performed to identify and classify the strains. A distribution different from that reported by Reid et al. (1996) was obtained. The dominance of *L. delbrueckii* subsp. *delbrueckii* and *L. acidophilus* in the homofermentative group was also different from that reported by Lachlack et al. (1996), probably because of ecological reasons of different geographical areas. The frequency of *Lactobacillus* isolation in our study was not

Table 3. Percentage of lactobacilli isolates with basic and acid characteristics.

Level	Chloroform				Ethyl acetate			
	OHO	FHE	OHE	Total	OHO	FHE	OHE	Total
Low	22	7	12	41	26	16	14	55
Medium	10	12	2	23	17	10	4	30
High	23	8	5	36	13	1	1	15

Low: strains with partition in chloroform and ethyl acetate from 0 to 35%. Medium: from 36 to 70%. High: from 71 to 100%. The method is described in the text.

Table 4. Adhesion properties of selected lactobacilli.

Strain	Hydrophobicity (%)			HA[b]	SAT[c]
	Hexadecane	Xylene	Toluene		
L. acidophilus F59[a]	96	82	82	A	+
L. acidophilus F93	73	84	89	ABO	A
L. crispatus F117	92	70	83	ABO	A
L. delbrueckii subsp. *bulgaricus* F37	78	35	35	ABO	A
L. delbrueckii subsp. *delbrueckii* F65	89	90	90	O	A
L. delbrueckii subsp. *delbrueckii* F86	92	78	77	O	+
L. delbrueckii subsp. *delbrueckii* F80	96	91	95	ABO	A
L. delbrueckii subsp. *delbrueckii* F91	89	86	76	—	+
L. delbrueckii subsp. *delbrueckii* F108	81	87	87	—	A
L. delbrueckii subsp. *delbrueckii* F73	93	88	88	ABO	+
L. delbrueckii subsp. *lactis* F78	75	91	91	ABO	+
L. delbrueckii subsp. *lactis* F28	77	60	56	ABO	A
L. fructosus F55	87	84	88	ABO	−
L. gasseri F85	89	83	80	—	+
L. gasseri F106	86	89	81	ABO	A
L. gasseri F104	87	86	81	—	A
L. jensenii F52	83	65	85	—	+
L. jensenii F74	78	74	87	O	A
L. salivarius F35	95	71	75	—	+
L. salivarius F71	95	75	91	O	A
L. agilis F54	85	88	75	ABO	−
L. agilis F61	74	95	95	O	A
L. agilis F71	95	75	91	O	A
L. agilis F99	86	96	81	—	A
L. rhamnosus F92	83	86	86	—	A
L. paracasei subsp. *tolerans* F3	90	77	97	ABO	−
L. coryniformis subsp. *coryniformis* F84	86	90	89	—	A
L. paracasei subsp. *paracasei* F2	72	85	78	ABO	A
L. paracasei subsp. *paracasei* F28	77	60	56	ABO	A
L. plantarum F47	96	78	77	—	A
L. brevis F68	83	88	83	—	A
L. brevis F56	83	83	83	—	+
L. brevis F69	74	88	86	ABO	A
L. buchneri F38	86	81	71	—	A
L. fermentum F53	86	85	91	—	A
L. reuteri F26	95	84	52	O	+

[a] F accounts for the internal nomenclature.
[b] HA: hemagglutination test, capital letters (A, ABO, and O) indicate the blood group that aggregates with lactobacilli.
[c] SAT: Salt Aggregation Test, A: self-aggregative, +: positive, −, negative.

very high, probably because obligate anaerobic lactobacilli were left out.

Some surface characteristics of the bacteria were studied to infer adhesion properties. The ability of bacteria to adhere to tissues is considered an important factor in the colonization of the different human and animal environments. Bacterial adherence has been suggested to be the result of two essentially different mechanisms: specific and nonspecific binding (Piette and Idziak, 1992). Nonspecific binding involves electrostatic or hydrophobic interactions of lower affinity than in specific binding. Piette and Idziak (1992) reported that cell-surface charge and hydrophobicity influence the strength of adhesion. The role of nonspecific hydrophobic interactions in bacterial adherence has led to the development of a wide variety of investigative methods: adherence to different plastic and glass materials, salting-out of bacteria with increasing concentrations of ammonium sulfate, and the extraction of bacteria from aqueous suspensions by mixing with immiscible hydrocarbons are just a few examples. Some of these techniques have been used for the comparison and prediction of adhesive properties of different types of bacteria (Marin et al., 1997). We used two to select lactobacilli. The original MATH method described by Rosenberg et al. (1983) and modified by Geertsema et al. (1993) was performed by using hexadecane as hydrophobic solvent. The modification of Sweet et al. (1987) included the use of xylene. Toluene was added as a third hydrophobic solvent. Our results showed a high degree of similarity between the three solvents employed, as shown by the correlation coefficients obtained. This means that under the same experimental conditions, they can be used indistinguishably to study the hydrophobicity of bacteria.

The other test employed to predict hydrophobic characteristics was SAT. This technique, introduced by Lindahl et al. (1981), is based on the premise that increasingly hydrophobic bacteria will aggregate at correspondingly lower salt concentrations (Rosenberg and Doyle, 1990). This method was previously used to ascertain the surface properties of many microorganisms, such as *Staphylococcus aureus* (Jonsson and Wadstrom, 1984). In our study, no correlation was found between the results obtained with this method and MATH.

Basic and acid surface characteristics of our isolates were studied by chloroform and ethyl acetate partition methods. Pelletier et al. (1997) found that strains with affinity for chloroform had no affinity for ethyl acetate, but they did for hexadecane (chloroform and hexadecane are both modulated by Van der Waals forces). Our results showed no strong correlation. The percentage of strains with a difference higher than 50% between chloroform and ethyl acetate was only 20%. Furthermore, because a tendency of bacteria to migrate to the basic/acid organic phase does not mean they lack affinity for the acid/basic solvent, no conclusions about the bacterial surface (acid or basic) can be assumed. Neither was a correlation found between partition with chloroform and hexadecane.

HA ability was another characteristic tested. This property is used as an adhesion test on the basis that the receptors of epithelial mammalian cells have a structure similar to that of erythrocytes. This ability was correlated with the presence of lectins on the surface of the bacteria (Piette and Idziak, 1992). These adhesion characteristics are shared by the uropathogenic *E. coli* strains that can hemagglutinate because of the presence of the proteinaceous P-fimbriae. A unique report of HA ability of lactobacilli is that of Andreu et al. (1995), who grouped them according to characteristic patterns. Among the strains studied, we found different degrees of HA. This test allowed us to select strains with HA ability for further studies.

The lack of correlation among the results of SAT, MATH, and HA ability of the microorganisms suggests that hydrophobic interactions do not play a major role in the ability of lactobacilli to agglutinate RBC, at least not under the test conditions. We can also infer that there are probably different structures and properties of the cell surface involved in each technique employed, which was expressed for SAT and MATH by Rosenberg and Doyle (1990).

Vaginal lactobacilli with the most promising properties were selected for further studies (Table 4). They will be tested for the production of antagonistic substances and adhesion to biological substrates and to epithelial cells. The absence of collateral effects when applied to animals and good technological properties (resistance to freezing, after freeze-drying survival) will also be tested to suggest the elaboration of a probiotic.

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