

Identification of *Lactobacillus* spp. in colostrum from Chilean mothers

Céline Dubos, Natalia Vega, Claudia Carvallo, Paola Navarrete, Carol Cerda, Oscar Brunser, Martin Gotteland

Lab. of Microbiology and Probiotics and Lab. of Biotechnology, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile

SUMMARY. The biodiversity of *Lactobacillus* spp. in colostrum samples from 116 Chilean mothers was analyzed by PCR and 16S rDNA sequencing. Lactobacilli were isolated in 55.3% of the samples, with concentrations of 3.33 ± 0.55 (log CFU/ml). The predominant species were *L. plantarum* (64%), *L. fermentum* (16%) and *L. pentosus* (9%). 28% of the isolated strains were resistant to gastric pH and bile salts, suggesting that they could be used as probiotics.
Key words: *Lactobacillus*, probiotics, breast milk.

RESUMEN. Identificación de *Lactobacillus* spp. en calostro de madres chilenas. Se analizó la biodiversidad de especies de *Lactobacillus* en muestras de calostro de 116 madres chilenas mediante PCR y secuenciación del rDNA 16S. Se aislaron lactobacilos en 55,3% de las muestras, con concentraciones de $3,33 \pm 0,55$ (log UFC / ml). Las especies predominantes fueron *L. plantarum* (64%), *L. fermentum* (16%) y *L. pentosus* (9%). 28% de las cepas aisladas fueron resistentes a pH gástrico y a las sales biliares, lo que sugiere que podrían ser utilizados como probióticos.

Palabras clave: *Lactobacillus*, probióticos, leche materna.

INTRODUCTION

During the first days of life, the establishment of the microbiota in the gastrointestinal tract of the newborn is mainly determined by the mode of delivery, vaginal or cesarean, and the mode of feeding, breast milk or formula (1). The colonic microbiota of the breastfed infant is characterized by the presence of *Bifidobacterium* and, in lower proportions, *Lactobacillus* which are considered as health-promoting microorganisms. Their growth in the newborn's colon is stimulated by a number of "microbial growth factors" present in the breast milk such as oligosaccharides, nucleotides and some proteins and bioactive peptides (2). In addition, recent evidence indicates that breast-milk is also a source of lactic acid bacteria including *Bifidobacterium* and *Lactobacillus* spp. (3,4). Due to the immaturity of the gastric barrier function, these microorganisms could be of importance for the newborn gut colonization. However it is important to note that most the studies evaluating the biodiversity of the breast milk microbiota have used low numbers of milk samples and that all of them have been carried out in mothers from European countries. Accordingly, the aim of this study was to evaluate the biodiversity of *Lactobacillus* spp. in breast milk samples obtained from Chilean mothers living in a low socio-economic district of Santiago, Chile. In addition, as breast milk has been proposed as a source of possible probiotics (5,6), we also evaluate the resistance of the isolated strains to gastric pH and bile salts.

MATERIALS AND METHODS

The study protocol was accepted by the Ethics Committee of INTA, Univ. of Chile and a written informed consent was obtained from the mothers previous to their participation. 116 healthy mothers of at-term infants and without any pharmacological treatments were recruited at Hospital Luis Tisne Maternity Ward located in a low-socioeconomic area in South East of Santiago. Two-milliliter samples of colostrum were collected aseptically in duplicate by a Registered Nurse during the first two-days of the postpartum. The samples were maintained at 4°C until their processing in the Gastroenterology Laboratory of INTA (at the most 2 h. after their emission). They were diluted in PBS buffer and 100 µL of the dilution were spread on MRS agar and incubated anaerobically for 48h at 37°C. Colonies corresponding to gram-positive, non-motile and indol- and catalase-negative bacteria were used for genotyping. Bacterial DNA were extracted from 1ml of the overnight culture and subsequently amplified by PCR using the following primers: 27F: 5' AGA GTT TGA TCM TGG CTC AG 3' and 1492R: 5' TAC GGY TAC CTT GTT ACG ACT T 3' (7). The PCR products were checked in 1% agarose gel, purified and subsequently sequenced. For the identification of the *Lactobacillus* species, sequences were compared with those from the Ribosomal Database Project (<http://rdp.cme.msu.edu/>).

Resistance to gastric pH conditions and to bile salts was evaluated by incubating the *Lactobacillus* strains at 37°C in

saline solution containing 0,32% pepsin (W/V) and adjusted to pH 2,0 with HCl for 90 min., or by incubating the strains at 37°C in MRS broth containing 0.4% Oxgall for 24h, respectively. Strains were considered as resistant when their initial concentration did not decrease more than one logarithm at the end of the incubation periods. Results of gastric and bile salt resistance between species were compared by Chi-square.

RESULTS

Presumptive *Lactobacillus* spp. were detected in 64 of the 116 colostrum samples (55.3%). As some samples had more than one type of colony, 127 isolates were finally obtained and 100 of them could be subsequently sub-cultured and identified by PCR and 16S rDNA sequencing. In the samples containing *Lactobacillus*, the concentrations of these microorganisms were 3.33 ± 0.55 log CFU/ml of colostrum. Results corresponding to the *Lactobacillus* identification are shown in Table 1: the predominant species were *L. plantarum* followed by *L. fermentum* and *L. pentosus* while *L. casei/paracasei*, *L. brevis*, *L. rhamnosus* and *Leuconostoc lactis* were found in proportions lower than 5% of the isolates. The evaluation of the gastric and bile salt resistance indicates that only 28% of the isolated strains were resistant to these conditions, without differences between species.

DISCUSSION

Our results confirm that breast milk is a source of *Lactobacillus* strains which may possibly impact the newborn's gut colonization process and exerts health-promoting effects. A great diversity of microorganisms has been described in the human milk; some of them originate from the skin, such as *Staphylococcus* and *Streptococcus*, while the origin of lactic

acid bacteria such as *Lactobacillus*, *Lactococcus*, *Enterococcus* and even *Bifidobacterium* spp. remains unclear. Some reports suggest that these microorganisms might be brought to the lactating breast tissue through the endogenous trafficking of bacteria-loaded dendritic cells originated from the intestinal mucosa (8). Our results indicate that these microorganisms may be detected early after delivery as the colostrum samples we used were obtained during the first two-day postpartum. This may explain why *Lactobacillus* strains were not found in all samples (only 55% of them) and why their concentrations were slightly lower than those previously reported in other studies (3). Studies using culture-based methods for evaluating *Lactobacillus* species in human milk mainly detected the presence of *L. gasseri* (3,9). Other studies carried out in a low number of breast milk and using DNA-based methods suggest the presence of other species such as *L. rhamnosus*, *L. plantarum* and *L. fermentum* (10). Our study confirms these results as we observed that *L. plantarum*, *L. fermentum* and *L. pentosus* were the predominant species found in the colostrum samples in this study, while *L. casei/paracasei*, *L. brevis* and *L. rhamnosus* were found in lower proportions. The former are not usual inhabitants of the human gut but it is probable that they may colonize temporarily the gastrointestinal tract of newborns due to their immature gastric acid secretion. The presence of bile salts may be a limiting factor for such colonization but our results show that 28% of the strains isolated from the colostrum samples were resistant to the bactericidal activity not only of adult gastric pH but also of bile salts. Accordingly, it is probable that these strains may be important for the newborn's health, contributing to reduce the risk of infection by entero-pathogens and to modulate the immune response of host as shown in previous studies (5,6).

TABLE 1
Frequency of isolation of *Lactobacillus* spp from breast milk from Chilean mothers and resistance of the isolated strains to gastric pH and bile salts

<i>Lactobacillus</i> species	pH and bile salt resistant strains	pH and bile salt sensitive strains	Total (%)
<i>L. plantarum</i>	17	47	64
<i>L. plantarum</i> / <i>L. pentosus</i>	2	7	9
<i>L. fermentum</i>	7	9	16
<i>L. casei</i> / <i>L. paracasei</i>	2	3	5
<i>L. brevis</i>	0	4	4
<i>L. rhamnosus</i>	0	1	1
<i>Leuconostoc lactis</i>	0	1	1
Total	28	72	100

REFERENCES

1. Adlerberth I, Wold AE. Establishment of the gut microbiota in Western infants, *Acta Paediatr* 2009; 98: 229-238.
2. Gotteland M, Cruchet S, Brunser O. Functional Food in Child Nutrition. In J Smith and E Charter Eds. *Functional Food Product Development*; Blackwell Publishing Ltd; 2010; p 440-458.
3. Martín R, Langa S, Reviriego C, Jiménez E, Marín M, Xaus J. et al. Human milk is a source of lactic acid bacteria for the infant gut. *J Pediatr* 2003;143:754-55.
4. Martín R, Jiménez E, Heilig H, Fernández L, Marín ML, Zoetendal EG, Rodríguez JM. Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR. *Appl Environ Microbiol* 2009; 75: 965-969.
5. Diaz-Ropero MP, Martín R, Sierra S, Villoslada L, Rodríguez JM, Xaus J, Olivares M. Two *Lactobacillus* strains isolated from breast milk differently modulate the immune response. *J Appl Microbiol* 2006; 102:337-343.
6. Olivares M, Diaz-Ropero MP, Martín R, Rodríguez JM, Xaus J. Antimicrobial potential of four *Lactobacillus* strains isolated from breast milk. *J Appl Microbiol* 2006; 101: 72-79.
7. de Long EF. Archaea in coastal marine environments. *Proc Natl Acad Sci USA* 1992; 89: 5685-5869.
8. Donnet-Hughes A, Perez PF, Doré J, Leclerc M, Levenez F, Benyacoub J et al. Potential role of the intestinal microbiota of the mother in neonatal immune education. *Proc Nutr Soc* 2010; 69: 407-15.
9. Matsumiya Y, Kato N, Watanabe K, Kato H. Molecular epidemiological study of vertical transmission of vaginal *Lactobacillus* species from mothers to newborn infants in Japanese, by arbitrarily primed polymerase chain reaction. *J Infect Chemother* 2002; 8: 43-49.
10. Martín R, Heilig HG, Zoetendal EG, Jiménez E, Fernández L, Smidt, H, Rodríguez JM. Cultivation-independent assessment of the bacterial diversity of breast milk among healthy women. *Res Microbiol*. 2007;58:31-37.

Recibido: 09-12-2010

Aceptado: 20-01-2011