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Viability and biological properties of probiotic vaginal lactobacilli after lyophilization and refrigerated storage into gelatin capsules

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Abstract

In the present study we investigated the survival and stability of probiotic properties of three vaginal lactobacilli after freeze drying with the addition of individual and combined compounds commonly used as pharmaceutical excipients and/or starters cryoprotectants, and subsequent refrigerated storage during 15 months. Lactobacilli, suspended in lactose, skim milk, ascorbic acid and combinations of them, were freeze-dried, packed into gelatin capsules and stored at 5 °C under darkness. At regular intervals throughout storage, freeze-dried samples were rehydrated and viability, adhesion to vaginal epithelial cells and antimicrobial activities were evaluated. The *Lactobacillus* tested conserved high viability up to 12 months in capsules containing ascorbic acid or combined excipients whereas lyophilization and storage with lactose or skim milk significantly decreased their survival. Abilities to produce lactic acid, H_2O_2 and bacteriocin were affected to different extents depending on the condition assayed. Lyophilization and storage also reduced the capacity of lactobacilli to adhere to vaginal epithelial cells but this property was partially restored after the first subculture in broth. The results obtained suggests that suitable selection of excipients that could also act as protecting agents of LAB during storage should be a valuable step in the development of a probiotic formulation with a stable shelf-life.

Keywords: *Lactobacillus*; Vagina; Probiotic; Survival; Lyophilization; Storage

1. Introduction

Lactobacilli are the most prevalent and numerically dominant microorganisms of the vaginal bacterial microbiota and they play a major role in the maintenance of a healthy urogenital tract by preventing the overgrowth and invasion of potentially pathogenic bacteria [1]. Several mechanisms such as competitive exclusion and displacement of uropathogens, production of hydrogen peroxide, organic acids, bacteriocins and the release of biosurfactants have been involved in their protective effect [1–4]. In consequence, a disruption of the population balance and particularly a depletion of vaginal lactobacilli has been associated with an increase in genital and urinary infections [5,6]. Accordingly, the probiotic use of lactobacilli as a non-chemotherapeutic mean to restore and maintain a normal vaginal flora and prevent disease recurrence has gained wide interest over the last years and represents a promising alternative [4] to conventional therapies.

Our research group has previously isolated and identified vaginal lactobacilli from healthy women of Tucumán in Argentina [7]. The strains were extensively characterized for their probiotic and technological properties and some interesting characteristics such as adhesion, auto and co-aggregation abilities, hydrogen peroxide, bacteriocin-like substances and organic acids production were reported [8–11]. Relevant technological properties, for instance, the optimal conditions for the production of antimicrobial substances were also determined for selected strains [12–14].

Medications for vaginal application are conventionally manufactured as creams, gels, tablets, capsules and ovules. At present, several probiotic vaginal products are commercially available; however the majority of them are unreliable in their content (the *Lactobacillus* species advertised in the label) and viable counts [15].

From a technological point of view, a probiotic formulation should include selected microorganisms with the ability to survive at high levels during the industrial process and remain viable afterwards with unaltered properties for long periods of storage (shelf-life). Stability after conservation and storage has been extensively studied in microorganisms commonly used as

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starters in the food industry [16–18]. In a previous study we have observed a short-term survival of vaginal lactobacilli included into glycerinated gelatin ovules [19]. On the contrary, Maggi et al. [20] have determined that several vaginal lactobacilli strains maintained a high viability for at least 18 months when incorporated as lyophilized powders into a tablet formulation.

Lyophilization is a process extensively used for preservation and long-term storage of biological samples. However, during freeze-drying the cells experience extreme environmental conditions such as low temperature and low water activity that produce structural and physiological injury to the bacterial cells resulting in the loss of viability of many species [17]. To prevent or reduce these undesirable side effects, protective substances are commonly added to the samples before freezing or freeze-drying [16,21,22].

Since probiotic preparations (both food and pharmaceutical formulations) include besides the "active substance" (the probiotic culture) different additives or excipients (emulsifying, antioxidants, preservatives, etc.), it would be technologically and economically valuable to evaluate the effect of these compounds on the physiology of the selected microorganisms. For this reason, the aim of the present study was to evaluate the survival rates and probiotic properties of three human vaginal lactobacilli lyophilized with different individual and combined substances (commonly used as pharmaceutical excipients) and stored into gelatin capsules during a period of 15 months.

2. Materials and methods

2.1. Microorganisms and growth conditions

Three *Lactobacillus* strains originally isolated from vaginal swabs of healthy women [7] were selected for this study. *Lactobacillus acidophilus* CRL (Centro de Referencia para Lactobacilos Culture Collection) 1259 releases inhibitory amounts of lactic acid against urogenital pathogens [14]; *L. paracasei* subsp. *paracasei* CRL 1289 is an hydrogen peroxide producer [8,13] and *L. salivarius* CRL 1328 releases a bacteriocin-like substance to its environment [9]. The human uropathogenic strains: *Escherichia coli* and *Enterococcus faecalis* used as sensitive strains for antimicrobial tests were provided by the Institute of Microbiology "Luis Verna" of the University of Tucumán, Argentina. Before experimental use, all the strains stored in milk-yeast extract at −20 °C were propagated in LAPTg broth [23]: 1.5% (w/v) peptone, (Difco Laboratories, Detroit, MI, USA), 1% tryptone (Difco), 1% glucose (Britania Laboratories, Buenos Aires, Argentina), 1% yeast extract (Difco) and 0.1% Tween 80 (Sigma Chemicals, St. Louis MO, USA), pH 6.8. The microorganisms were incubated at 37 °C and subcultured at least twice in this medium every 12 h.

2.2. Cells production and storage conditions

Lactobacilli at mid-exponential phase of third subculture were inoculated at a rate of 2% (v/v) into 1 L of LAPTg broth and incubated without shaking at 37 °C for 12 h. Cells, at early stationary phase, were harvested by centrifugation (10,000 × *g*, 10 min, 4 °C), washed twice with sterile distilled water, and concentrated ten-fold (100 ml). This suspension was fractioned into seven flasks (15 ml), centrifuged and finally resuspended to the original volume (15 ml) into the following individually and combined substances: 8% lactose (Anedra, Buenos Aires, Argentina), 6% skim milk (Nestlé, Buenos Aires, Argentina) and 2.5% ascorbic acid (ICN Biomedicals Inc., OH, USA). The suspensions obtained, with a cell density of about 5×10^9 colony-forming units per ml (CFU ml^{-1}), were frozen at −80 °C, desiccated under vacuum for 18 h (LYOBAC GT-2 freeze-drier equipment) and the lyophilized powders were placed aseptically into empty gelatin capsules (Parafarm™, Drogueria Saporiti SACIFIA, Buenos Aires, Argentina) (50 mg/capsule approximately). Table 1 shows the combination of excipients applied for the seven conditions tested. The capsules were placed in glass flasks containing silica-gel desiccants and stored at 5 °C under darkness. Viability, antimicrobial substances production and adhesion abilities were determined at defined time intervals throughout a storage period of 15 months.

Table 1
Composition of the capsules designed to evaluate the survival rates of probiotic vaginal lactobacilli[a]

Base (gelatin capsules)	Active principle (lactobacilli, 10^9 CFU g^{-1})	Excipients		
		Lactose (8%)	Milk (6%)	Ascorbic Acid (2.5%)
Condition 1	+	+	−	−
Condition 2	+	−	+	−
Condition 3	+	−	−	+
Condition 4	+	+	+	−
Condition 5	+	+	−	+
Condition 6	+	−	+	+
Condition 7	+	+	+	+

[a] Lactobacilli were suspended in individual and combined excipients, freeze-dried and stored into gelatin capsules as described in Section 2.

The assays were performed with microorganisms obtained from the lyophilized powders rehydrated in 1 ml of saline solution (0.9% NaCl) at 25 °C and those obtained after the first subculture in LAPTg broth. Antimicrobial activities were determined in microorganisms obtained only from the latter condition.

2.3. Viability of lactobacilli

The number of viable lactobacilli before and after freeze-drying and storage was determined by a plate count method. Lyophilized powders were rehydrated in 1 ml of sterile saline solution (0.9% NaCl) at 25 °C and these suspensions were serially 10-fold diluted in sterile peptone-water (0.1% peptone, Difco). All dilutions were poured into LAPTg supplemented with 1.5% agar and colonies were enumerated after incubation of plates at 37 °C for 48 h. Results were expressed as log of CFU g^{-1} of lyophilized powder. The weight of freeze dried microorganisms was determined by difference between full and empty capsules.

2.4. Antimicrobial substances production

The antimicrobial substances production was quantified in the spent supernatant fluids of the first subculture of bacteria in LAPTg broth incubated at 37 °C for 12 h (early stationary phase). Inhibitions of *E. coli* by lactic acid produced by *L. acidophilus* CRL 1259 and *E. faecalis* by the bacteriocin produced by *L. salivarius* CRL 1328 were determined according to the plate diffusion techniques previously described [9,14]. The hydrogen peroxide produced by *L. paracasei* subsp. *paracasei* CRL 1289 grown under agitation was determined by the *o*-dianisidine horseradish peroxidase spectrophotometric modified method [24].

2.5. Adherence assay

Adhesion assays were basically performed as described by Ocaña et al. [10]. Suspensions of lactobacilli coming from lyophilized powders and from the first subcultures were washed with sterile saline solution and resuspended in Eagle's Minimal Essential Medium (MEM; Gibco) (adjusted at pH 4 with lactic acid) to obtain aprox. 5×10^6 CFU ml^{-1} to 1×10^7 CFU ml^{-1}. Vaginal epithelial cells (VEC) were collected in MEM at pH 4 by scraping the vaginal walls of healthy volunteers with a cytobrush (Cyto Soft Brush, Medical Packaging Corp., USA). Indigenous bacteria were removed by washing cells with MEM (120 × *g*, 10 min) and the concentration of vaginal epithelial cells was finally adjusted to

10^5 cells ml^{-1}. Suspensions of lactobacilli and VEC were mixed (1:1) and incubated at 37 °C for 1 h in microaerophilic conditions. After incubation, each suspension was centrifuged (120 × *g*, 10 min), the pellet was resuspended in 10 ml of MEM and then filtered through an 8 μm-pore size membrane (Millipore, Corp., Bedford, MA, USA) in order to remove all the non-adhering bacteria. Membranes containing VEC with adhered lactobacilli were washed under agitation in Petri dishes with 10 ml of saline solution. Serial ten-fold dilutions of these suspensions were poured into LBS agar (Rogosa agar, Merck) and plates were incubated under microaerophilic conditions for 48 h at 37 °C. The colonies were enumerated and adhesion percentages were calculated according to the following expression: adhesion index = (log CFU adhered - bacteria/log CFU total bacteria) × 100.

2.6. Data analysis

Two separate trials were conducted for each strain and the results presented are the average of duplicate samples taken at each time. The non-parametric Kruskall–Wallis test was used to measure statistical differences between means at the 5% level of significance.

3. Results

3.1. Survival of vaginal microorganisms after freeze-drying and storage into gelatin capsules

No relationship was found between the excipients used and the survival of microorganisms after lyophilization. Immediately after freeze-drying, the viability of the three strains tested declined between 0.05 and 2 log cycles in all the conditions assayed (Fig. 1).

However, the use of different compounds singly or combined as suspending media of bacterial cells, significantly increased the survival of *Lactobacillus* during the storage period. The survival of *L. acidophilus* CRL 1259, *L. paracasei* CRL 1289 and *L. salivarius* CRL 1328 after the lyophilization process and at regular intervals of 3 months during storage are shown in Fig. 2. As a general result, ascorbic acid used individually or combined with lactose, milk or both as the drying medium for lactobacilli, acted as a protective agent and improved the survival of microorganisms during the storage in a significant manner ($p < 0.05$). In these conditions, the three *Lactobacillus* under study conserved high viability for at least 12 months. On the other hand, microorganisms lyophilized and stored with single lactose or skimmed milk significantly declined ($p < 0.05$) since the first months of storage.

L. acidophilus CRL 1259 retained a high number of viable cells in capsules containing freeze-dried bacteria with ascorbic acid alone or combined with milk, lactose or both, showing a decrease in the viable counts of 1 log cycle or less at 12 months of storage (Fig. 2A). Viability in capsules containing lyophilized microorganisms with lactose or milk remained steady up to the third month of storage but gradually decreased after that period. However, a marked decline was observed in all the conditions tested between 12 and 15 months of storage and no survivors were detected in capsules containing milk or lactose as excipient by the end of the sample period (Fig. 2A). A survival percent of 100% (9.7 log CFU g^{-1}) after 12 months of storage was found in capsules containing milk + ascorbic acid ($p < 0.05$) whereas at 15 months, less than 4% of initial microorganisms were recovered from all conditions containing ascorbic acid.

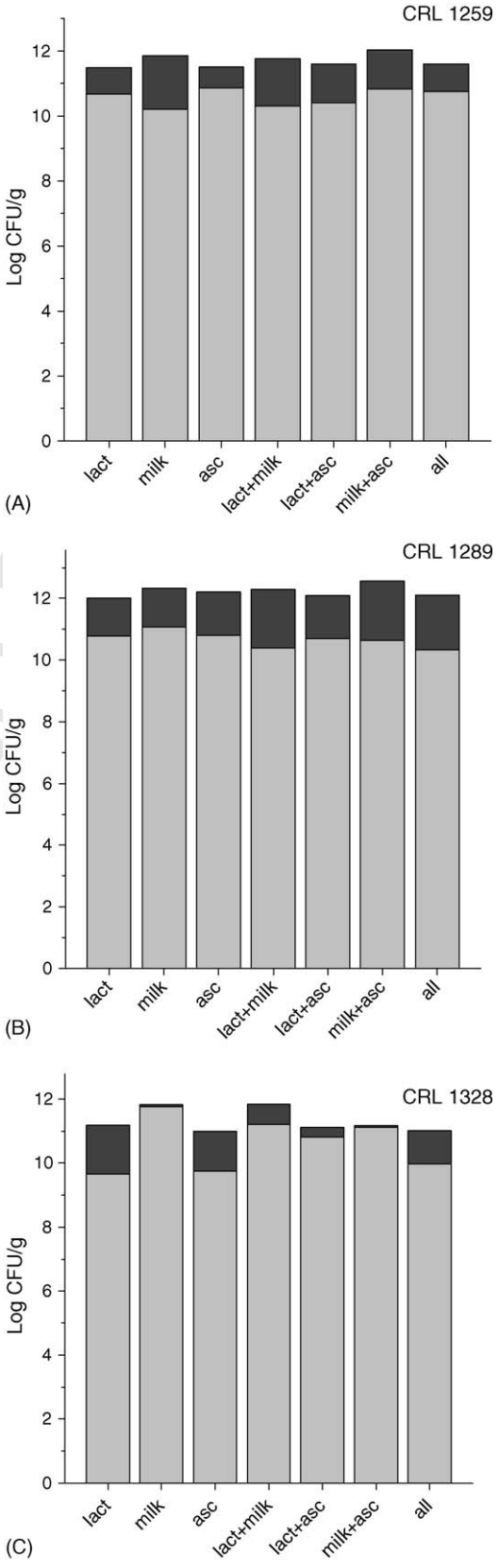

Fig. 1. Survival (log CFU g^{-1}) of vaginal lactobacilli before and after freeze drying with different excipients. (A) *Lactobacillus acidophilus* CRL 1259, (B) *L. paracasei subsp. paracasei* CRL 1289 and (C) *L. salivarius* CRL 1328. (■) Before, (▩) after.

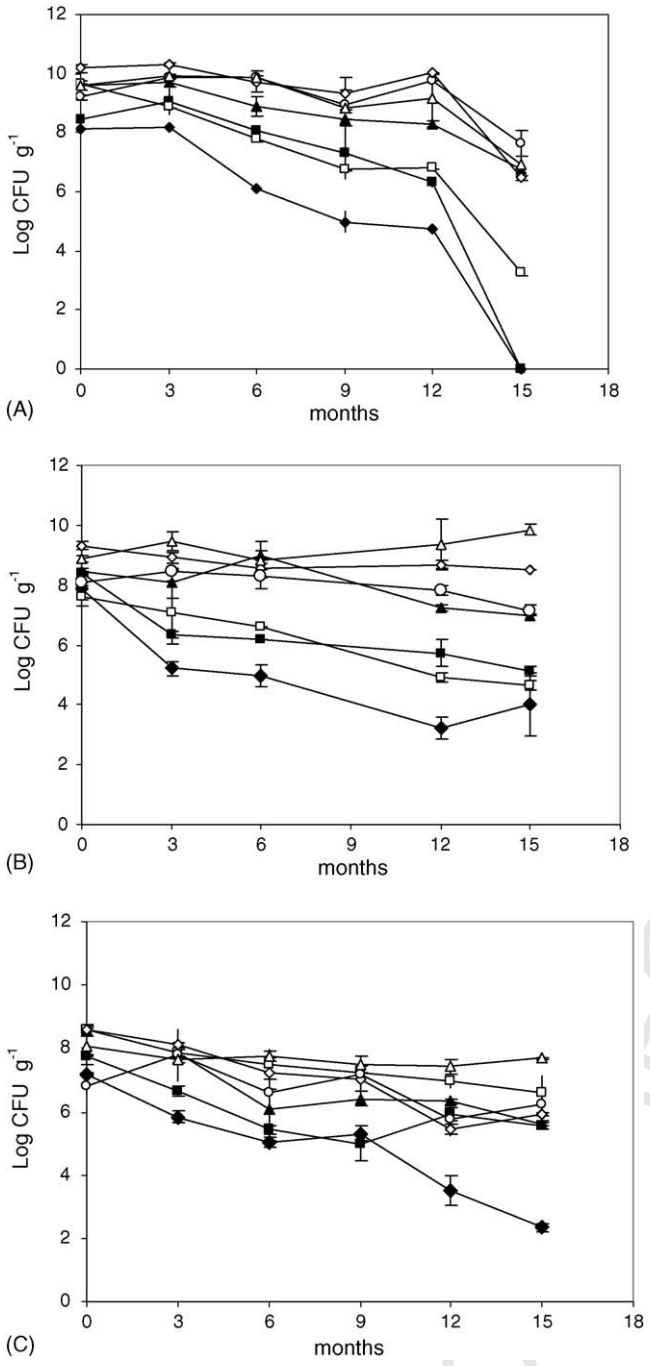

Fig. 2. Survival (A–C) of probiotic vaginal lactobacilli stored as freeze-dried powders into gelatin capsules containing different excipients during 15 months at 5 °C. (A) *Lactobacillus acidophillus* CRL 1259; (B) *L. paracasei* subsp. *paracasei* CRL 1289; (C) *L. salivarius* CRL 1328. The results reported are the means ± standard deviation of the data (log CFU g^{-1}) obtained from duplicate samples of two independent assays. (◆) Lactose; (■) milk; (▲) ascorbic acid; (□) lactose + milk; (○) lactose + ascorbic; (◇) milk + ascorbic; (△) lactose + milk + ascorbic acid.

In the case of *L. paracasei* CRL 1289, decreases of 2 and 3 log cycles were obtained at 3 months of storage in capsules containing lyophilized lactobacilli with milk or lactose respectively, and no abrupt decline was observed at any of the conditions tested beyond month 12 (Fig. 2B). Capsules with lactobacilli suspended in milk + lactose + ascorbic acid showed the best storage condition, since no decrease in the viable counts was observed, and 100% of the original cells number was recovered after 15 months of storage (9.8 log CFU g^{-1}).

The survival of *L. salivarius* CRL 1328 decreased progressively to different extents in all the conditions tested. At 15 months of storage only the capsules containing the three combined excipients, retained a higher population than the other conditions (7.6 log CFU g^{-1}) ($p < 0.05$) (Fig. 2C). The greatest reduction was observed in capsules containing lactose with a survival decrease of aroun 5 log cycles (from 7.2 to 2.3 log CFU g^{-1}). For this particular strain, the suspension of bacteria in milk + lactose also preserved, to a lesser extent, the viability of cells during storage.

3.2. Effect of freeze-drying and storage on antimicrobial activities

Antimicrobial activities were evaluated before and after freeze-drying (0 months) and at some intervals during storage (6 and 15 months) (Table 2). The production of antimicrobial substances and their ability to inhibit pathogenic bacteria depend on the growth status of the producer: *L. acidophilus* CRL 1259 showed a scarce growth at 12 h of incubation in the first subculture of lactobacilli coming from capsules stored 6 and 15 months with individual excipients and milk + lactose (10^3 CFU ml^{-1} approximately). As a consequence, there was no enough lactic acid produced in these supernatants to inhibit uropathogenic *E. coli* (Table 2). In the case of *L. paracasei* CRL 1289 and *L. salivarius* CRL 1328, the first subcultures of the seven conditions tested were at the early stationary phase of growth ($\cong 5 \times 10^7$ CFU ml^{-1} to 2×10^8 CFU ml^{-1}) when the antimicrobials were determined. Results showed that the production of H_2O_2 by *L. paracasei* CRL 1289 was decreased by freeze-drying and storage in bacteria coming from capsules with lactose, milk and both, but was not affected or even increased in the other conditions tested (Table 2). For *L. salivarius* CRL 1328, the bacteriocin synthesis was not affected by the lyophilization in any of the conditions assayed but was slightly decreased because of the storage in capsules containing single lactose, being completely abolished in the microorganisms recovered from capsules with ascorbic acid.

3.3. Adhesion capabilities

Fig. 3 shows that both lyophilization (0 months) and storage (6 and 15 months) affected the adhesion capabilities of the three vaginal lactobacilli to different extents. However, no relationship was found between the excipients used and adhesion indexes. After 15 months of storage, no adhesion was observed in the rehydrated powders obtained from some conditions of the three strains. As a general pattern, adhesion indexes were lower in microorganisms obtained from rehydrated powders than in those coming from the first subculture in LAPTg broth. The latter values were, in some cases, even higher than the control values of microorganisms routinely cultured in broth (e.g. adhesion of *L. salivarius* CRL 1328 at 0 and 6 months) (Fig. 3). These results suggest that immediately after rehydration, most

Table 2
Effect of lyophilization (0 months) and 6 and 15 months of storage on antimicrobial substances production by probiotic vaginal lactobacilli

	L. acidophillus CRL 1259[a]; lactic acid producer; presence of inhibition haloes			*L. paracasei* CRL 1289[b]; H_2O_2 producer (mmol/L)			*L. salivarius* CRL 1328[c]; bacteriocin producer (AU/ml)		
	0	6	15	0	6	15	0	6	15
Lactose	+	−	Nd	1.37	0.02	0.11	533.3	533.3	400.0
Milk	+	−	Nd	1.51	0.63	0	533.3	533.3	533.3
Ascorbic acid	+	−	−	4.60	7.39	4.50	533.3	0	0
Lactose + milk	+	−	−	3.79	0.80	1.51	533.3	533.3	533.3
Lactose + ascorbic	+	+	+	4.76	5.94	4.67	533.3	533.3	533.3
Milk + ascorbic	+/−	+	+/−	5.58	7.49	4.92	533.3	533.3	533.3
Lactose + milk + ascorbic	+	+	+	5.23	4.61	4.03	533.3	533.3	533.3

The assays were performed with the spent supernatant fluids of the first subculture of bacteria in LAPTg broth incubated at 37 °C for 12 h.
[a] Control of *E. coli* inhibition by lactic acid produced by *L. acidophilus* CRL 1259 at third subculture (pre-lyophilized). +: Clear inhibition haloe of ≥6 mm produced by $\leq 10^7$ CFU ml^{-1}; +/−: inhibition haloe of ≤6 mm; −: no inhibition haloe. Not determined (no growth of microorganisms).
[b] Control of H_2O_2 production by *L. paracasei* CRL 1289 at third subculture (pre-lyophilized): 4.65 ± 0.72 mmol/L (produced by $\cong 10^7$ CFU ml^{-1}).
[c] Control of bacteriocin synthesis by *L. salivarius* CRL 1328 at third subculture (pre-lyophilized): 533.33 AU/ml (produced by $\cong 10^8$ CFU ml^{-1}).

bacteria are diminished in their ability to bind to epithelial cells and that adhesion abilities were partially restored after the subculture of lyophilized microorganisms. The particular behavior of each strain can be summarized as follows:

- *L. acidophillus* CRL 1259: Both lyophilization and storage decreased adhesion to VEC but adhesion abilities were completely restored by first subculture in all conditions at 0 months. At 6 and 15 months of storage, the adhesiveness was completely and partially restored, respectively, in microorganisms coming from capsules with combined ascorbic acid.
- *L. paracasei* CRL 1289: At 6 months of storage, adhesion abilities were partially restored after the first subculture only in bacteria coming from capsules with individual excipients but was restored in all conditions tested at the end of sample period.
- *L. salivarius* CRL 1328: After lyophilization and 6 months of storage, adhesion to VEC increased significantly by first subculture to values even higher than the control in the seven conditions tested. At 15 months, adhesion was restored to control value in all the conditions assayed.

4. Discussion

Since the production process could be critical for preserving viability and biological properties of microorganisms, a suitable selection of excipients that could also act as protecting agents of LAB should be recommendable in the development of probiotic formulations. Recently, we have determined that ovules prepared with a water-soluble base such as glycerinated gelatin provides only a short-term survival of probiotic vaginal lactobacilli which could be prolonged by the use of ascorbic acid [19]. As another vehicle and delivery form that ensure a long term survival of LAB should be selected, we assessed in the present study the viability and biological properties of three human vaginal lactobacilli after freeze drying with some compounds (commonly used as excipients) and subsequent storage into gelatin capsules during 15 months.

Survival rates of the microorganisms varied among the strains and the agents used as the suspending media. For the three microorganisms studied, their suspension in ascorbic acid individually or combined with milk, lactose or both exerted a protective effect and enhanced their survival during storage. Our results agree with other studies on the viability of different bacterial species commonly used as food starters after freezing, freeze-drying and refrigerated storage [17,21,22,25,26]. It has been reported that different polyols, amino acids, sugars and components of culture media can exert a protective effect [21,25,27] by inhibiting the intracellular formation of ice, membranes damage, protein denaturation, etc., and decreasing therefore the cells injury. Milk components and sugars as lactose and sucrose have shown to be effective protectors for *Lactobacillus* species and *Bifidobacterium* during freeze-drying and storage [16,22,25] whereas the higher survival of dried *Enterococcus durans* and *E. faecalis* could be related to their exposure to media components such as Tween 80 and ascorbic acid [27]. In the present study, neither lactose nor milk protected vaginal lactobacilli from progressive cellular death during storage whereas their combination only partially protected the cells of *L. salivarius* CRL 1328. The application of combinations of protective substances led to a better survival than the obtained with excipients individually used [22], being ascorbic acid mixed with milk and lactose the best storage condition for freeze-dried vaginal lactobacilli. Some hypotheses have been proposed to explain the mechanisms underlying the protection afforded by the components used in our and other studies. It is supposed that milk favors bacterial survival at low temperature by stabilizing the cell membrane constituents and forming a protective coating on the cell wall proteins [21]. Lactose could act as an effective protector due to the presence of hydroxyl groups which provide protection against free radicals and by their water binding capacity that prevents intracellular ice formation [25]. Ascorbic acid is an antioxidant agent and its effectiveness as protector seems to be related to the inhibition of membrane lipids oxidation which in turn affects the survival of cells during freeze drying and their subsequent storage in the dried state [26].

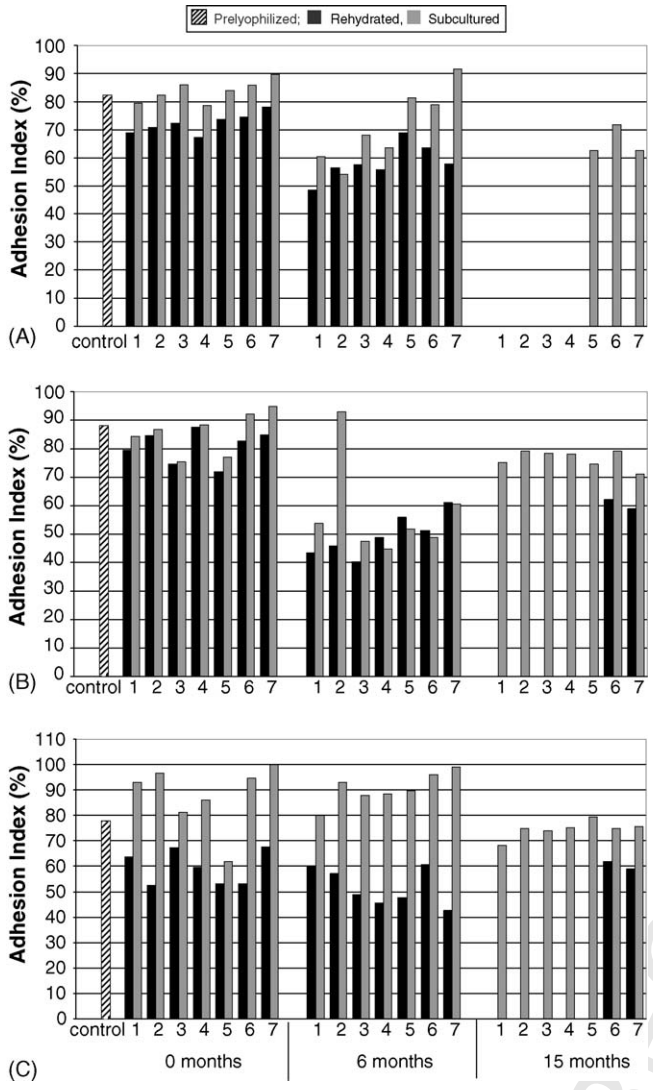

Fig. 3. Influence of lyophilization (0 months) and storage (6 and 15 months) on adhesion of *L. acidophilus* CRL 1259 (A), *L. paracasei* CRL 1289 (B) and *L. salivarius* CRL 1328 (C) to vaginal epithelial cells. Adhesion was determined at pH 4 with rehydrated (■) and subcultured microorganisms (▩). Control values (▨) were determined at the third subculture of each strain on LAPTg broth incubated at 37 °C for 12 h. Adhesion index: log CFU adhered bacteria/log CFU total bacteria × 100. Nd: not determined (no growth of microorganisms).

As previously mentioned, in addition to maintaining the viability of cultures, it is also important that their probiotic properties remain unaltered following the freeze-drying process and storage. The results obtained do not allow any generalization since each strain showed a particular behavior, but mixtures of excipients and those preferably including ascorbic acid favored the maintenance of antimicrobial activities after a long-term storage. Since the antimicrobial properties were preserved after storage in similar conditions for the three strains (and it is known they do not inhibit one to each other) it should be optimum to combine them into a probiotic formulation with a wider action spectrum against uropathogenic microorganisms.

Adhesion to epithelial cells favors colonization of the host and persistence in the target organ for time enough to exert the probiotic effects. Therefore, the influence of processing and storage on the adhesive properties of the selected microorganisms is an important factor to take into consideration. Maggi et al. [20] demonstrated that addition of polymers to a tablet formulation enhanced the adhesion of some vaginal strains to VEC but significantly reduced adhesiveness in others, whereas Mastromarino et al. [28] suggested that compounds capable of reducing the surface negative electric charges added to tablets manufacture would greatly improve the colonization capacity of vaginal lactobacilli. In this way, any excipient used for pharmaceutical formulations should be tested on the adhesion capacity of both probiotic lactobacilli and prevalent pathogenic bacteria of the geographic area under investigation. In the present study both lyophilization and storage affected adhesiveness of vaginal lactobacilli with a greater decrease at longer storage periods. However, adhesion capabilities were significantly recovered after subcultivation of the microorganisms in growth medium. Mastromarino et al. [28] hypothesized that lyophilization could probably modify the conformation of surface bacterial adhesins, but after first culture the adhesion capacities were restored to the level achieved with routinely cultured microorganisms. Since our results could suggest an inverse relationship between the time of storage and ability to colonize the vagina in vivo, it should be important to select excipients that, besides their protective effect on the bacterial cells, could stimulate lactobacilli growth in order to restore adhesiveness. In this sense, Reid et al. [29] have proposed that substances such as skim milk, ascorbic acid, vitamins and lactose applied vaginally could alter the urogenital flora by stimulating lactobacilli growth in preference to pathogens. Further studies are also needed to elucidate if there is any relationship between adhesion capabilities and viability of the bacterial cells.

5. Conclusions

Ascorbic acid added to the microbial suspensions before drying was found to favoured the stability of the cells during long term storage, and therefore should be considered for the production of freeze-dried cultures that will eventually be included as probiotics in pharmaceutical preparations. Since lyophilization and storage can affect the antagonistic activities of lactobacilli against pathogens and their adhesion capabilities, these factors must be taken into account in the product preparation and for each individual strain it must be investigated if their properties are maintained after processing. The probiotic strains used in this study retained, in the appropriate conditions, high viable populations and their probiotic properties for long periods of storage. Further studies are actually in course in order to select other suitable excipients and the best delivery form and vehicle for administering these lactobacilli to the host in clinical trials designed to test their protective and therapeutic effects against urogenital infections.

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