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Research Paper

Alginate Films Containing Viable *Lactobacillus Plantarum*: Preparation and *In Vitro* Evaluation

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Abstract. The objective of the study was to develop calcium alginate films, containing *Lactobacillus plantarum* ATCC 8040 with preserved and stable viability and antibacterial activity. *L. plantarum*-loaded films containing different calcium concentrations were physically characterized for mechanical and bioadhesive properties and lactobacilli release. The viability and antibacterial activity of *L. plantarum* was studied before and after processing, and during 6 months of storage. A multiresistant clinical isolate, VIM-2-metalo-β-lactamase producing *Pseudomonas aeruginosa*, was used as an indicator strain. Interference between *L. plantarum* and films enhanced films elasticity, water absorption ability, release of lactobacilli, and decreased films adherence. A decrease of *L. plantarum* viability in alginate films (≤1 log unit) was observed after freeze drying. *L. plantarum*, at cell concentrations of 10^8 cfu/ml, was inhibitory active. The viability and antibacterial activity of the immobilized lactobacilli remained stable during 6 months of storage. The study has proved the potential of alginate films to deliver *L. plantarum* in high numbers to individuals.

KEY WORDS: alginate film; antibacterial activity; bacterial viability; immobilization; lactobacilli.

INTRODUCTION

Lactobacilli are probiotic bacteria, considered as Generally Recognized as Safe organisms (1), which when administered in adequate amounts confer health benefit on the host (2). Application of lactobacilli to replace pathogenic bacteria on skin and mucosa is an attractive approach for prevention and treatment of infections (3). Moreover, the use of lactobacilli could be of particular interest for burn wound treatment (4–6). Burn patients treated with *Lactobacillus plantarum* culture showed the same recovery as patients treated with silver sulphadiazine, suggesting that lactobacilli could be used for topical treatment of burns (5). In addition, we have shown that alginate films loaded with *L. plantarum* have a large potential on prevention of burn wound infections (6). The development of such an application however, depends strongly on the preservation technologies applied in the processing of bacteria.

Consequently, the aim of the present study was to optimize the technological conditions in the production of alginate films containing *L. plantarum* with preserved and stable viability and antibacterial activity, suitable for topical application. *L. plantarum* ATCC 8014 was chosen among lactobacilli strains based on its activity against multi-resistant clinical isolates including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumoniae* (7) and on its ability to withstand adverse conditions during processing into delivery systems (8). A VIM-2-metalo-β-lactamase producing *P. aeruginosa* FFUL 1396 (6) was used as an indicator strain. *P. aeruginosa* is a prevalent organism in burn patients, resulting in severe complications due to its high frequency of antibiotic resistance (9). Calcium alginate was chosen to design the delivery system of *L. plantarum* as a polymer widely used in burn wound treatment due to its haemostatic, gel-forming and granulation-encouraging properties (10). Moreover, according to our earlier experiments (7), lactobacilli immobilization in alginate beads was favorable in preservation of viable and antibacterially active lactobacilli.

MATERIALS AND METHODS

Materials

Glycerol, sodium chloride, and calcium chloride (Sigma-Aldrich, Chemie, Germany), disodium hydrogen phosphate and potassium dihydrogen phosphate (Pancreac, Barcelona, Spain), ethylenediaminetetraacetic acid (EDTA, Merck, Darmstadt, Germany), sodium alginate (Protanal LF 10/60, FMC BioPolymer, Drammen, Norway), de Man–Rogosa–Sharpe agar and broth (MRS), and Cetrimide agar (Biokar Diagnostics, France) were used.

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Methods

Bacterial Strains and Culture Conditions

L. plantarum ATCC 8040 was grown at 37°C for 18 h on MRS agar and in broth. A VIM-2-metalo-β-lactamase-producing *P. aeruginosa* FFUL 1396, isolated from abdominal wound of a patient at a Central Hospital in Lisbon, was grown overnight at 37°C for 18 h on its selective agar medium Cetrimide. The stock cultures produced at the maximum cell growth were maintained at −80°C in growth medium (0.850 ml) supplemented with glycerol (0.150 ml).

Preparation and In Vitro Evaluation of L. Plantarum-Loaded Calcium Alginate Films

Preparation of L. plantarum-loaded calcium alginate films. Sodium alginate (1 g) was dissolved in *L. plantarum* overnight cultures (50 ml). Of the cell–alginate suspension, 9.5 ml was poured into a Petri plate (5.5 cm diameter) containing 3 ml of twofold-increasing $CaCl_2$ concentrations (0.05–12.8 M). The films were cured for 15 h at 4°C in the calcium solutions to reach steady-state equilibrium (11). Ellipse-shaped films were obtained. The time of film formation was recorded and film dimensions were measured with calipers (Dias de Sousa, Portugal; Table I). As the method of preparation by pouring influences the films dimensions, film strips (dimensions 3.2×2.2×0.5 cm) were cut out to obtain thickness uniformity across the surface at different points of the film.

After curing, each film was incubated into MRS broth (50 ml) at 37°C/4 h to obtain proliferation of the entrapped lactobacilli to the films surfaces (7). As we have observed previously (7), both the alginate matrix and the effective networks by the mutual contact of surface and entrapped lactobacilli promote high survival rates during freeze drying. Blank films, containing only MRS broth and no lactobacilli, were used as controls.

All types of films were frozen at −80°C for 24 h and dried under vacuum at room temperature, 0.035 mbar for 24 h (Christ Alpha 1–4, B. Braun Biotech International, Germany). The films produced were kept over silica gel, at 4°C, for a maximum period of 6 months. These films were considered for further characterisation, including scanning electron microscopy, films thickness, calcium content, water absorption capacity, mechanical properties, bioadhesive properties, release of lactobacilli, and lactobacilli viability and antibacterial activity (Table II).

Scanning electron microscopy. Samples from the lactobacilli-loaded and blank films were mounted on metal stubs with double-sided adhesive tape and coated with gold (JEOL JSM-1200, Japan) under vacuum and 15 mA at room temperature. The morphology of coated samples was examined by scanning electron microscopy (JEOL JSM-5200 LV, Japan).

Calcium content. The calcium concentration in the films is of paramount to their formation and physicochemical properties. Therefore, amount of calcium remaining in the film provides information of the properties of the films. The calcium content in the prepared films was measured by atomic absorption analysis (Perkin Elmer AAnalyst–700, USA). A series of standard calcium chloride solutions (0, 1, 2, 4, 6, 8, and 10 mg/L) was prepared from 0, 0.5, 1, 2, 3, 4, and 5 ml calcium stock solution (100 mg/L), 10 ml EDTA solution (37.7 g/L), and deionized water up to 50 ml. Samples (100 mg) from films were dissolved in sodium citrate solution (1%, pH 6.0) and then diluted in EDTA solution (7.5 g/L) and in deionised water. The calcium content in the samples was determined by comparison with standard solutions.

Water absorption capacity. Films (dimensions 3.2×2.2×0.4 cm) were suspended in 50 ml of phosphate-buffered saline (PBS; pH 7.4) at room temperature. PBS was prepared by mixing of 1 ml of phosphate buffer (pH 7.5; 0.33 M) with 29 ml of sodium chloride (9 g/L) and final pH adjustment. At specified time intervals (ranging between 1 min and 48 h, depending on the film), the films were taken out and the excess of water removed carefully with filter paper, then weighed immediately. The swelling percentage of the films was calculated by the equation:

$$\frac{Ws - Wd}{Wd} 100 \tag{1}$$

where, *Ws* is the weight of the swollen sample and *Wd* is the weight of the dry sample.

Mechanical properties. The mechanical properties of film strips (3.2×2.2×0.4 cm) were measured using a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) equipped with a 5 kgf (~50 N) load cell and fit with a three-point bending rig apparatus. Film strips were hydrated for 5 min to allow comparison between different films, and placed over the supports, positioned at a distance of 7 mm. The upper blade moved down equidistant from the two lower supports at a speed of 3 mm/s. The breaking force was measured and the tensile strength (12) and Young's modulus (13) were calculated as follows:

$$\sigma_f = \frac{3Fl}{2bd^2} \tag{2}$$

$$E = \frac{Fl^3}{4\xi d^3 b} \tag{3}$$

Table I. Visual Inspection of the Forming Films (mean ± SD), Before Drying

$CaCl_2$ formation solution (M)	0.8	1.6	3.2	6.4	12.8
Time for film formation (h)	3	2.5	1	1	0.25
Film major and minor axis (mm)	42.5±0.7/47.4±1.2	43.4±1.3/46.0±1.9	43.3±1.2/46.0±1.3	43.0±1.3/45.0±1.5	43.0±1.2/45.0±1.3
Film thickness (mm)	5.23±0.26	5.08±0.19	5.08±0.19	4.65±0.19	4.35±0.15

Table II. Physical Characteristics of Calcium Alginate Films (mean ± SD), After Drying

	$CaCl_2$ formation solution [M]	Film calcium content (mM)	Thickness (mm)	Swelling ratio	Crushing force (N)	Tensile strength ($\times 10^3$ N/m^2)	Young's modulus ($\times 10^3$ N/m^2)	Work of boiadhesion ($\times 10^{-2}$ J/m^2)	Peak detachment ($\times 10^{-2}$ N/m^2)
Lactobacilli loaded films	0.8	0.91	4.1±0.29	10.07±0.12	0.84±0.047	23±4	8±3	53±5	156±45
	1.6	1.65	4.0±0.12	8.96±0.58	1.09±0.28	35±11	15±2	55±5	145±45
	3.2	2.37	4.0±0.12	8.06±0.62	1.38±0.24	42±7	23±1	56±5	155±38
Blank films	0.8	1.55	3.85±0.11	8.96±0.20	1.33±0.24	44±8	18±1	67±13	87±31
	1.6	3.22	3.84±0.20	7.05±0.61	1.35±0.28	45±4	20±5	50±6	65±11
	3.2	4.55	3.85±0.25	3.62±0.25	1.53±0.25	48±11	24±3	119±28	270±52

where, σ_f is the tensile stress at fracture, F is the load at fracture, l is the distance between the supports, b is the film width, d is the film depth, E is Young's modulus, and ξ is the deflection at the midpoint.

Bioadhesive properties. The bioadhesive strength of the films was evaluated according to Sezer *et al.* (14). The measurement was conducted with a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) fit with a 50 N load cell and bioadhesion test apparatus which included a cylindrical Perspex probe. Chicken back skin was used as a model tissue, after the removal of all fats and debris. The dermal tissue was fitted on the bioadhesion test apparatus, and then 100 μl of demineralized water was applied on the surface of the tissue prior to measurements at 37°C. The film was cut into small cylinders shape and attached to probe with double-sided adhesive. The probe was lowered onto the surface of the tissue with a constant speed of 1 mm/s and constant force of 1 N applied. After keeping in contact for 30 s, the probe was then moved vertically upwards at a constant speed of 1 mm/s. Work of adhesion (Joules per square meter) and peak detachment force (Newton per square meter) were calculated from the force *versus* displacement graphic.

Release of lactobacilli. Samples of films (100 mg) were placed into Falcon tubes (50 ml) containing 10 ml of PBS (pH 7.4) and then placed into a shaking water bath at 37°C (20 osc/min). At specified time periods (10, 30, 60, 120, 270, 360, and 600 min), 100 μl from every test tube were withdrawn and replaced with fresh PBS. Serial dilutions were made in sterile water and plated in duplicate on MRS agar to allow counting of lactobacilli released. Percentage of bacteria released was calculated from initial film loading ($8.5\times10^9\pm1.41\times10^8$ cfu/g, $8.0\times10^9\pm2.12\times10^8$ and $8.0\times10^9\pm2.83\times10^8$ cfu/g for films obtained with 0.8, 1.6, and 3.2 M $CaCl_2$, respectively).

Viability of lactobacilli. The lactobacilli viability was determined before and after immobilization and freeze drying and throughout 6 months of storage by the agar plate method. Of the samples, 20 mg was collected from the center and the periphery of films. These results were averaged for each sample and then the mean and standard deviation calculated: low standard deviation reflected little variation between the center and the periphery of the film. Calcium alginate was dissolved in sterile sodium citrate solution (1%, pH 6.0) followed by serial dilutions made in sterile water and plated in duplicate on MRS agar.

Antibacterial activity. Micro- and macrodilution broth methods (7) were used to test the *in vitro* antibacterial activity of *L. plantarum* before and after processing into calcium alginate films and during 6 months of storage. *L. plantarum* at concentrations of 10^8 to 10^2 cfu/ml was co-incubated with *P. aeruginosa* (10^5 cfu/ml). Samples were analyzed at 6, 8, 10, and 24 h to determine the time necessary for the inhibition of *P. aeruginosa*.

Statistical analysis. Significance of results was found by performing one-way ANOVA tests followed by a Tukey post hoc test to the data (Statistical Package for Social Sciences, SPSS Int. BV, USA, v.17.0).

RESULTS

The production of a film was observed with a minimal calcium concentration of 0.8 M $CaCl_2$. Below this value, films did not show an adequate structure falling apart. On the other hand, by increasing the concentration of calcium in the calcium chloride solution, the time for films formation decreased (Table I) together with a smaller volume observed (before freeze drying, Table I; and after freeze-drying, Table II).

The aqueous solution of $CaCl_2$ used in a concentration up to 3.2 M did not affect significantly the *L. plantarum* viability. However, high concentrations of $CaCl_2$ (6.4 M) led to a lack of uniformity in *L. plantarum* viability, *i.e.*, *L. plantarum* cell concentration in the periphery of the film (2×10^7 cfu/ml) was lower compared to the cell concentration in the central part of the films (8×10^8 cfu/ml; higher standard deviation bars; Fig. 1a, immobilization) and to a viability decrease lower than the limit of detection noted with 12.8 M $CaCl_2$ (Fig. 1a, immobilization). Freeze drying resulted in a decrease of approximately 1 log unit: from 4.7×10^9 to 2.4×10^8 cfu/ml ($p=0.0382$), 3.3×10^9 to 3.0×10^8 cfu/ml ($p=0.0012$), 1.8×10^9 to 3.0×10^8 cfu/ml ($p<0.0001$) of *L. plantarum* viability in films obtained with 0.8, 1.6 and 3.2 M $CaCl_2$, respectively. Stable viability of bacteria during 6 months of storage (monitored at 0, 3, and 6 months of storage) was observed in the same films (4.7×10^9 to 5.5×10^9 cfu/g ($p=0.5648$), 5.9×10^9 to 1.4×10^9 cfu/*g* ($p=0.1402$), 5.9×10^9 to 2.7×10^9 cfu/g ($p=0.1714$); Fig. 1b).

Lactobacilli-loaded films were characterized by smooth surface morphology, while blank films by rough (Fig. 2) with no significant differences on their thicknesses. The calcium content in films (Table II) was well below from the amount applied in film formation in both blank and lactobacilli-loaded films. The presence of lactobacilli led to a 1.70- to 1.95-fold decrease of the calcium concentration compared to blank films. The percentage of swelling of the lactobacilli-loaded films (705.7–906.6%) was significantly higher ($p<0.0001$) than the swelling percentage of blank films (261.6–795.6%). The water absorption capacity increased significantly with the decrease of calcium content, *i.e.*, the swelling percentage of the lactobacilli-loaded films formed with low calcium concentration (0.8 M) was significantly greater than loaded films formed with 1.6 M ($p=0.0201$) or 3.2 M ($p=0.0052$). By analogy, significant differences in the swelling percentage were observed among the blank films ($p<0.01$ to $p<0.0001$). The tensile strength values of the lactobacilli-loaded films ranged from 23 to 42×10^3 N/m^2 ($p=0.0625$), whereas the blank films showed smaller variations of the tensile strength values (44–48×10^3 N/m^2; $p=0.8679$). The presence of lactobacilli had a significant effect on crushing force and tensile strength, whereas the effect of the calcium content was only significant when lactobacilli were present. Similarly, Young's modulus values in *L. plantarum*-loaded films ranged from 8 to 23×10^3 N/m^2 ($p=0.0119$) and showed smaller variation in blank films (18–24×10^3 N/m^2; $p=0.1751$; Table II). The tensile

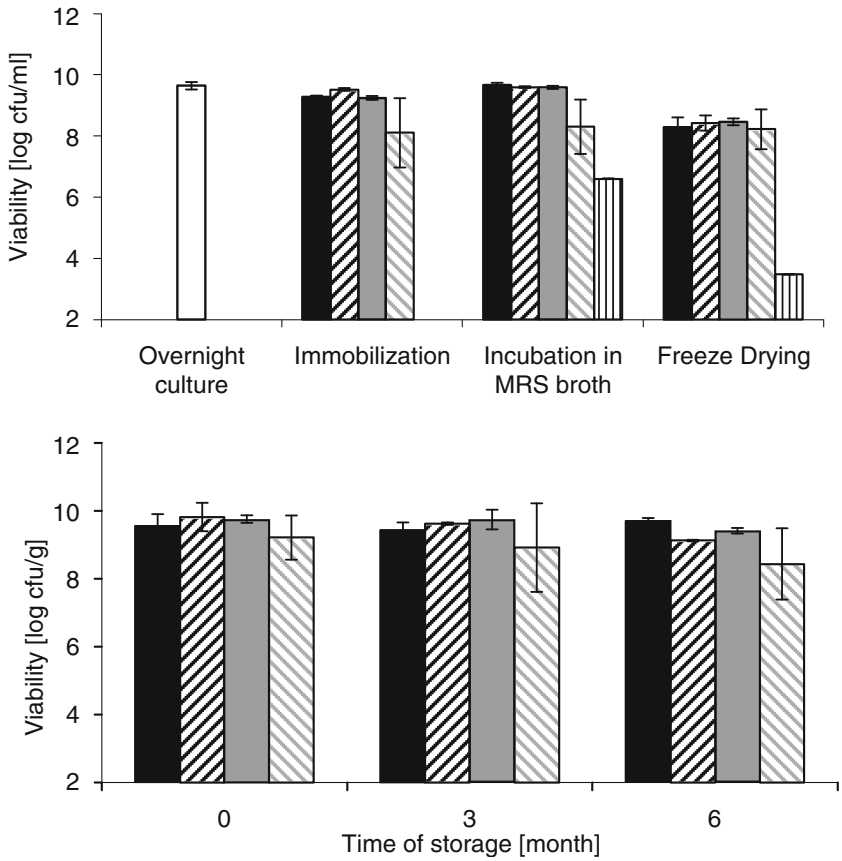

Fig. 1. Viability of *L. plantarum* (mean and SD, $n=3$) in calcium alginate films obtained with 0.8 M $CaCl_2$ (*black bar*), 1.6 M $CaCl_2$ (*dashed black bar*), 3.2 M $CaCl_2$ (*gray bar*), 6.4 M $CaCl_2$ (*dashed gray bar*) and 12.8 M $CaCl_2$ (*striped black bar*) before and after processing **a** and throughout 6 months of storage at 4°C **b**

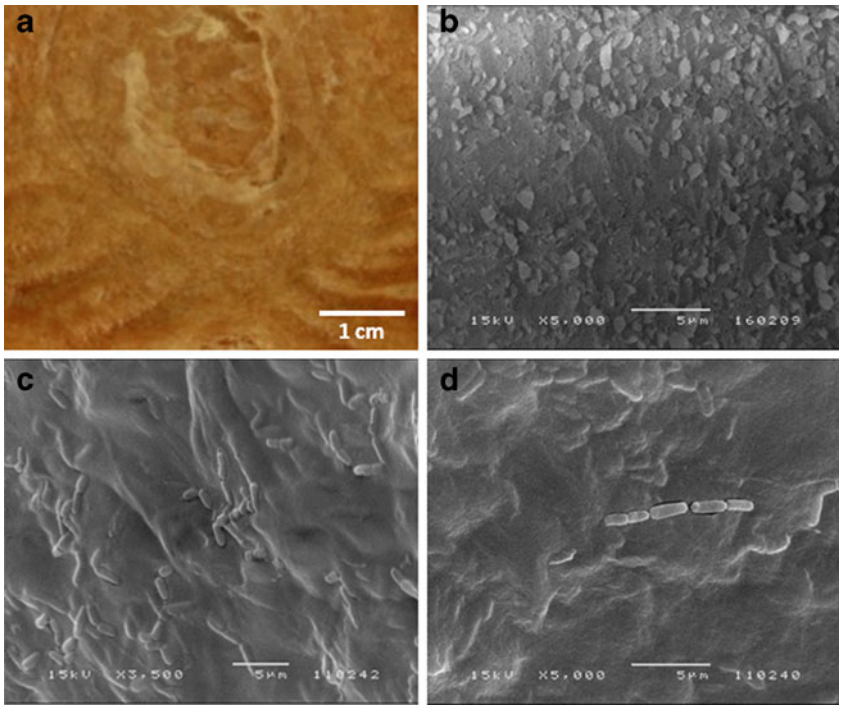

Fig. 2. Photo of loaded film **a** and scanning electron photomicrographs of internal surface **b** and *L. plantarum* loaded external **c** and internal **d** surface alginate films produced with 1.6M $CaCl_2$

strength as well as the Young's modulus of films obtained with 0.8 M calcium solutions differed significantly from films obtained with 3.2 M solutions ($p=0.0152$) and from the blank films. Thus, the increase of calcium content in films was reflected by an increase in tensile strength and decrease of elasticity of films. The films bioadhesion values ranged from 50 to 67×10^{-2} J/m^2, except with 3.2 M blank patch (119×10^{-2} J/m^2) which was significantly different from the other values (<0.0001; Table II). Significant differences were observed for the force required to detach the patches. Differences were dependent particularly on the presence of lactobacilli.

The lactobacilli released in the first 10 min from 0.8 and 1.6 M films corresponded to 10^8 cfu/g (1.59–1.88%) *vs.* 10^7 cfu/g (0.38%) released from the 3.2 M films. In 2 h, the released lactobacilli from the three types of patch corresponded to 10^9 cfu/g (35.2%, 23.1%, and 18.1%, respectively). Of the films, 0.8, 1.6, and 3.2 M released their total number of lactobacilli as follows: 4, 8, and 10 h, respectively (Fig. 3).

The nonprocessed *L. plantarum* cultures were active against *P. aeruginosa* at a cell concentration of $1.1\times10^7\pm2.9\times10^6$ cfu/ml. After the immobilization of *L. plantarum* into alginate freeze-dried films, as well as during storage, the inhibition of *P. aeruginosa* was obtained by $6.2\times10^7\pm5.4\times10^7$ *L. plantarum* cells/ml ($p=0.1764$). Inhibition of *P. aeruginosa* was observed 8 h after its co-incubation with nonprocessed or processed to films *L. plantarum* cultures.

DISCUSSION

When the cell–alginate suspension was added to $CaCl_2$ solutions, calcium alginate films were formed. These gel systems evolved slowly with time because of cross-linking reactions, taking place during the gel formation, proceeding progressively from the surface to the interior (11). The increase of calcium content reduced the time of film formation and allowed the building of a stronger and more compacted network (Table I). The observed decrease in film volume could be due to network formation, characterized by higher complexing between the carboxylate groups of the guluronate monomers of alginate and the calcium cations, resulting in reduced space occupied by alginate and therefore decreased film volume (11).

Figure 1 shows the viability of *L. plantarum* during film production and storage. The amount of calcium added to the film was critical to both film formation and lactobacilli viability. The higher the amounts of calcium, the higher the tensile strength of the film but lower the number of viable bacteria. Figure 1a shows that immobilization with high concentrated

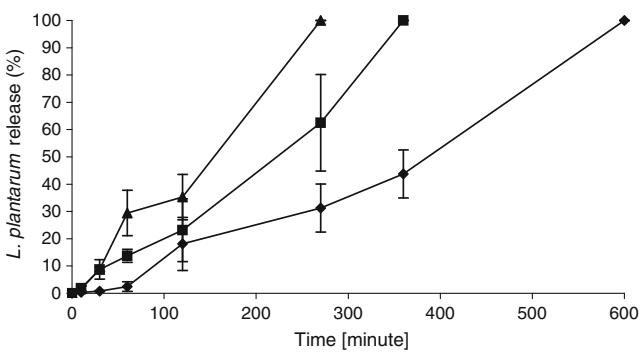

Fig. 3. Release of lactobacilli from films (formed with ▲ 0.8 M; ■ 1.6M; ● 3.2M $CaCl_2$), represented as % as a function of time

calcium solutions (6.4 and 12.8 M) was reflected by a decrease in the number of bacteria. The intracellular concentration of calcium in bacteria is tightly regulated ranging from 100 to 300 nM (15), whereas the calcium concentration in bacterial growth media can be approximately 20 μM (16). Generally, extracellular calcium concentration higher than 10 mM is considered to be toxic to cells (17). In the present study, the millimolar range of calcium measured in the films (Table II), when higher than 4.55 mM, resulted in a decreased *L. plantarum* viability. The hypertonic calcium solutions used in the film formation could have provoked plasmolysis with the cytoplasm shrinking and the plasma membrane contracting from the cell wall as a result of internal water loss. Consequently, cells may survive and resume growth if favorable conditions are found, as observed during incubation in MRS broth of films formed with high concentrated calcium solutions (6.4 and 12.8 M; Fig. 1a; 18).

Beside the influence on lactobacilli, the calcium concentration may affect the proliferation of fibroblasts (19). It is known that when applied to wounds, the calcium ions from the calcium alginate fibers rapidly exchange with sodium ions from the exudate and the release of free calcium ions provides one of the essential factors in the clotting cascade. However, the elevated release of calcium ions (6.7–12.8 mM) by wound alginate dressings was found to inhibit the proliferation of fibroblasts and to be partly responsible for a cytotoxic effect on fibroblasts and epidermal cells in culture (19). The same authors showed that alginate dressings with calcium content up to 4 mM had the least cytotoxic effect andencouraged quicker wound closure (19). The calcium content in the lactobacilli loaded (0.91–2.37 mM) was in the recommended range.

The preservation of the films by freeze drying has shown a decrease in *L. plantarum* viability of about 1 log unit. It must be pointed out that films formed with calcium concentrations up to 3.2 M have shown the smallest decrease in numbers (1 order of magnitude), whereas for higher calcium contents a significant decrease was observed. Different survival rates in films during freeze drying might be due to the different initial lactobacilli concentration in films. In general, surviving fraction of the microorganisms increases with the increasing biomass concentration before freeze-drying, and this is attributed to the mutual shielding effect of the microorganisms against the severe conditions of the external medium (20). Furthermore, cellular damage induced by the hypertonic calcium solutions could have accumulated through the freezing and drying processes (21).

Once immobilization was carried out in the best conditions, films were stable overtime (Fig. 1b). It means that the process of drying was adequate to preserve the bacteria and the calcium alginate with nutrients from the MRS broth left after incubation provided a proper protection to the lactobacilli.

The morphology of the films with and without lactobacilli was quite different. Figure 2 shows the surfaces of blank and loaded films. It might be due to the interaction between the lactobacilli cells and the calcium alginate matrix. The rough internal and external surfaces of films were transformed into smooth surfaces during the proliferation of lactobacilli in calcium alginate films. The proliferation of lactobacilli resulted in a decrease of the calcium content in the films, which could be due to production of lactic acid by the bacteria during incubation of films in MRS broth, which competes with the alginate for calcium (22).

Measurement of calcium content in the films has shown that a large part of calcium was not used in film formation (amount applied *versus* amount quantified). A possible explanation could be that the upper layers of the films (the first to be formed) prevented calcium chloride to go underneath. Velings and Mestdagh (11) described a parabolic distribution in the calcium concentration in the calcium alginate gels, which is higher on the surface of the gel that is in contact with the formation solution than in the center of the gels.

A decrease of calcium in the network affected the physical properties of the films (Table II). For the same surfaces (3.2×2.2 cm), the film strips have shown different thicknesses showing clearly an increase in thickness when lactobacilli are present. This suggests that the network is not so compact, as for the blanks, derived from lower calcium content, as discussed. Furthermore, the creation of networks between lactobacilli during their proliferation to the films surface could have contributed to decreasing the films shrinkage during freeze drying (7).The smaller compaction as a consequence increases the absorption of water by the films: the blank films show a larger range of swelling percentage (261.6–795.6%) than loaded films (705.7–906.6%). Results of the force at crush and the tensile strength emphasize the effect of the presence of lactobacilli. Whereas the blank strips show closer range of results (not significantly different), for the loaded strips the largest tensile strength almost doubles the smallest one. Still with the mechanical properties of strips, the Young's modulus shows that blank strips are less elastic than the loaded ones. This suggests that the network formed by the calcium alginate was stronger and less elastic in the absence of lactobacilli. The calcium content did also affect the release of lactobacilli from films. Increased cross-linking density with calcium and the consequent decrease of porosity (17) resulted in slower release of bacteria (Fig. 3). The lactobacilli were at both the surface of strips and within the network (Fig. 2c, d). The presence of surface lactobacilli explained the observed burst effect: 0.3–2.5% of the total bacterial load was released immediately after contact with PBS.

The adhesion between the loaded film and chicken skin was reflected by the work of adhesion which was quite constant for loaded strips whereas for blank strips it varied considerably. The explanation must be formed at the surface of the strips. Figure 2 clearly shows differences at the surface of strips: loaded strips have a smooth surface covered with bacteria whereas the blank strips have rough surface. The asperities observed might have increased the surface of contact between the strip and the chicken's skin in a non-anticipating pattern. The force at detachment showed that loaded strips required generally a larger force than blank strips. However, the work of adhesion was smaller for loaded strips. It shows that bacteria promoted an easier detachment of films under tension, decreasing the adhesion work.

The inhibitory effect of *L. plantarum* ATCC 8040 against a VIM-2-metalo-β-lactamase producing *P. aeruginosa* was studied *in vitro*. The results have shown that *L. plantarum* cultures at high cell concentrations of 10^7–10^8 cfu/ml before and after immobilization into calcium alginate films were effective against the multiresistant *P. aeruginosa*. The results showed that alginate films could be a suitable matrix preserving both viability and antibacterial activity of lactobacilli.

CONCLUSIONS

The study has confirmed the possibility of immobilization of *L. plantarum* in calcium alginate films preserving the viability and antibacterial activity of *L. plantarum*.

The study has demonstrated interference between *L. plantarum* and the alginate films resulting in improved water absorption capacity and film elasticity, quicker lactobacilli release, and lower adherence. These properties could be advantageous in wound management, protecting the wound from trauma, absorbing excessive exudate, promoting the delivery of lactobacilli to compete with pathogens, and prevent infection.

Finally, films could be considered solid dosage forms which are easier to handle and deliver to patients than the starting cell cultures or other liquid dosage forms.

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