Antimicrobial properties of magnesium chloride at low pH in the presence of anionic bases

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Abstract. Magnesium is an element essential for life and is found ubiquitously in all organisms. The different cations play important roles as enzymatic co-factors, as signaling molecules, and in stabilizing cellular components. It is not surprising that magnesium salts in microbiological experiments are typically associated with positive effects. In this study with Listeria monocytogenes as a model organism, we focus however on the usefulness of magnesium (in form of MgCl₂) as a stress enhancer. Whereas MgCl₂ does not affect bacterial viability at near-neutral pHs, it was found to strongly compromise culturability and redox activity when cell suspensions were exposed to the salt at acidic pH. The principle was confirmed with a number of gram-negative and gram-positive species. The magnesium salt dramatically increased the acidity to a level that was antimicrobial in the presence of anionic bases such as phosphate, lactate, or acetate, but not TRIS. The antimicrobial activity of MgCl₂ was much stronger than that of NaCl, KCl, or CaCl₂. No effect was observed with MgSO₄ or when cells were exposed to MgCl₂ in phosphate buffer with a pH ≥ 5. Acid stress was reinforced by an additional, salt-specific effect of MgCl₂ on microbial viability that needs further examination. Apart from its implications for surface disinfection, this observation might support the commonly stated therapeutic properties of MgCl₂ for the treatment of skin diseases (with healthy skin being an acidic environment), and could contribute to understanding why salt from the Dead Sea, where Mg²⁺ and Cl⁻ are the most abundant cation/anion, has healing properties in a microbiological context.

Key words: bacteria, magnesium chloride, sodium chloride, pH, antimicrobial, acid stress

Many important findings about the effects of different salts and cations on bacterial viability were made in the first three decades of the 20th century which many considered to be the ‘golden age’ of this type of research. Cations were reported to exert a highly characteristic effect upon bacteria: low concentrations of given salts were reported to favor viability, whereas higher concentrations were associated with growth inhibition [1-3]. This effect was visualized by an optimum curve that generally held true for all cations; however, the concentrations at which the transition between

beneficial and toxic occurred seemed to vary greatly among the different cations. Salts such as NaCl or KCl, which are typically ‘favorable’ (for example, in growth media or physiological saline solution), were reported to be inhibitory in sufficiently high concentrations. Whereas the latter correlates with common scientific ‘gut feeling’ and the concept of osmotic stress, the same principle could be implied to suggest that even highly toxic substances such as HgCl₂, PbCl₂ and other heavy metals might have a stimulating effect on bacterial growth in sufficiently low concentrations. Although there was no clear explanation of this empiric observation [3], the effects of different cations and their specific efficiencies (both in regard to stimulation and inhibition) resulted in the quantitative assignment of ‘specific potency’ factors. Na⁺ served as a reference and was assigned a potency factor of 1. Examples of potencies that were reported include: K⁺ = 1.2, Mg²⁺ = 9.4, Ca²⁺ = 12, Mn²⁺ = 400, Zn²⁺ = 700, and Cd²⁺ = 3000 [4].

Whereas these studies focused on the direct effect of the different ions on bacterial viability, this study addresses their impact under different pH conditions and buffer systems. The project was motivated by previous findings that bacterial viability was much more strongly affected by desiccation in the presence of MgCl₂ compared with other salts [5], that magnesium salts have antiseptic properties in treatments involving Dead Sea salts, and that the effects of different salts on bacterial viability depended greatly upon pH. Whereas the presence of salts typically had no effect at a neutral pH (compared to a sample without salt), a slightly stronger effect was observed in the acidic range.

Listeria monocytogenes served as a model organism because of our laboratory’s interest in this food-borne pathogen, although Escherichia coli, Salmonella enterica var Typhimurium (isolated from human feces), Enterococcus faecium (isolated from vaginal excretions); where not indicated otherwise, strains were from the Facultad de Ciencias Biológicas, University of Concepción. All bacteria were grown on tryptic soy agar (TSA; Becton, Dickinson and Company, Le Pont de Claix, France) at 30°C. Liquid cultures were obtained by inoculating 15 mL of tryptic soy broth (TSB) into a 50 mL Falcon tube, shaken at a 45° angle for 18 hours, at 120 rpm and at 30°C. Cell density was measured in a spectrophotometer (TU-1810 Split Beam UV-VIS, Electronic Co Ltd, Shanghai, China) at 600 nm (OD₆₀₀) and adjusted to an OD₆₀₀ = 1.0 by addition of fresh medium. Aliquots of 1 mL were transferred into 1.5 mL microcentrifuge tubes, and centrifuged (5,000 rpm, 5 min), followed by careful removal of the supernatant.

### Materials and methods

#### Bacterial strains and growth conditions

The bacterial strains used for this study included Listeria monocytogenes (ATCC 19115), Staphylococcus aureus (ATCC 2913), Escherichia coli (K-12), Salmonella enterica serovar Typhimurium (isolated from human feces), Enterococcus faecium (isolated from vaginal excretions); where not indicated otherwise, strains were from the Facultad de Ciencias Biológicas, University of Concepción. All bacteria were grown on tryptic soy agar (TSA; Becton, Dickinson and Company, Le Pont de Claix, France) at 30°C. Liquid cultures were obtained by inoculating 15 mL of tryptic soy broth (TSB) into a 50 mL Falcon tube, shaken at a 45° angle for 18 hours, at 120 rpm and at 30°C. Cell density was measured in a spectrophotometer (TU-1810 Split Beam UV-VIS, Electronic Co Ltd, Shanghai, China) at 600 nm (OD₆₀₀) and adjusted to an OD₆₀₀ = 1.0 by addition of fresh medium. Aliquots of 1 mL were transferred into 1.5 mL microcentrifuge tubes, and centrifuged (5,000 rpm, 5 min), followed by careful removal of the supernatant.

#### Sample preparation and pH exposure

The bacterial cell pellet was resuspended in 500 μL of phosphate buffer (100 mM) followed by addition of 500 μL of either water or solutions of different salts (final salt concentrations of 10, 50, 150, 400, or 1,000 mM; final buffer concentration of 50 mM). Phosphate buffer was adjusted to pH values between 2 and 11. Alternatively, cells were resuspended in TRIS, acetate or lactate solutions.
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(final concentrations of 50 mM each) adjusted to a pH of 4 by the addition of NaOH or HCl. Cells were exposed to the different solutions without salt or supplemented with different salts for 20 min each. The salts used in this study were: MgCl₂ (cat. nr. BM-0970; Winkler Ltda., Santiago, Chile), NaCl (cat. nr. SO-1455; Winkler Ltda., Santiago, Chile), KCl (cat. nr. 1.04936.1000; Merck), MgSO₄ (cat. nr. MA-0980; Winkler Ltda.), and CaCl₂ (cat. nr. CA-0520; Winkler Ltda). After salt exposure, cells were harvested by centrifugation (5,000 g, 5 min) and resuspended in phosphate buffer (50 mM; pH7).

Cultivation on plates

Aliquots of undiluted cell suspensions were transferred into the top row of sterile, 96-well Nunclon™ plates (Nunc, Roskilde, Denmark). Dilutions were made by stepwise mixing of 10 µL of cell suspension with 90 µL TSB pre-aliquoted in the lower rows. All dilutions and transfers were made using multichannel pipettes to allow for a rapid sample processing. Volumes of 3 µL of the undiluted cell suspension and the different dilutions were spotted onto TSA Petri dishes, with the highest cell concentration in the top row of the grid. After brief drying, plates were incubated at 30°C for approximately 20 h. Images of growth patterns were taken with a digital camera (Scion Corporation, Japan), and visualized using the Gel-Pro analyzer program (Media Cybernetics, USA).

Redox activity

For the measurement of redox activity, WST-8 (GenScript, Piscataway, USA) and menadione (2-methyl-1,4-naphthoquinone; ACROS Organics, Geel, Belgium) were dissolved in water and DMSO respectively, to obtain stocks of 10 mM and 8 mM that were stored at -20°C. WST-8, menadione, and water were mixed in ratios of 9:1:10 to obtain a detection reagent that was pre-aliquoted in 20 µL volumes in black, flat-bottom, 96-well microtiter plates (cat. nr. 5530100; Orange Scientific; Braine-l’Alleud, Belgium). The reaction was started by addition of 180 µL of 10-fold-diluted cell suspensions (prepared previously for the cultivation analysis) using a multichannel pipettor. Diluted cell suspensions were used as controls; without dilution the signals from untreated control samples were obtained too quickly. After addition of cells and mixing by pipetting up and down several times, plates were immediately transferred to a TECAN F200-Pro plate reader (TECAN, Austria). Signals were measured at 450 nm every two min for a total of three hours. Before every measurement, the plate was shaken for five seconds (linear shaking, amplitude of 3).

Fluorescence microscopy

Cells were stained using the LIVE/DEAD® BacLight™ bacterial viability kit (L13152; Invitrogen, Carlsbad, California). Following the manufacturer’s instructions, SYTO9 and propidium iodide were each dissolved in 2.5 mL of sterile water and subsequently blended to obtain a 2× staining solution. Cell suspensions were stained for 15 min in the dark followed by filtration on black, 0.22 µm, Isopore polycarbonate filters (cat. nr. GTBP02500, Millipore, USA). Filters were placed on a slide using the mounting oil provided with the kit. Images were acquired from a fluorescence Olympus BX51 microscope using a 100× (UPlanFI, Olympus, USA) objective, FITC and PI fluorescence filter sets (ex485/20, em535/25 and ex540/20, em635/350, respectively), and a Cool SNAP-Pro Digital Kit camera (Media Cybernetics Inc., USA). The software used for visualization was Image-Pro Plus 5.1 (Media Cybernetics Inc., USA).

Chemical speciation and statistical analysis

Calculation of the chemical speciation of salts at different pH values was performed with the software “CHEAQS pro V. 2004.1” [10]. Statistical ANOVA and Tukey analyses were performed using statistical software GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, California, USA).

Results

Comparison of different salts

To compare the effects of different common salts on bacterial viability, Listeria monocytogenes was resuspended in phosphate buffer at pH7 and pH3.
Comparison of effect at different pHs

In a next step *L. monocytogenes* aliquots were resuspended in phosphate buffer (initial pH between 2 and 11) in the presence or absence of MgCl$_2$ (figure 2). In the absence of salt, the only sample where viability was compromised was at pH2, whereas culturability was comparable for all other pHs (figure 2A). In the presence of salt, no effect on culturability was seen for the samples with an initial buffer pH of 5 or higher. Of the samples with an initial buffer pH of 4 or lower on the other hand, MgCl$_2$ was associated with a marked reduction of survival. The effect can, in part, be explained by the salt-induced decrease in pH (values of buffer-salt mixtures are indicated in figure 2). On the other hand, culturability in the pH3 sample (without salt) was higher than in the pH4 sample with salt (pH of buffer-salt mixture = 3.3), suggesting a pH-independent salt effect of unknown nature. The same observation applies when comparing culturability of the pH2 sample (without salt) with the one of the pH3 sample with salt (pH of buffer-salt mixture = 2.5). A more detailed insight was obtained by measuring redox activity (figure 2B): whereas the presence of salt did not affect activities in the pH range 6 and 9, a positive effect (more activity) was seen for pH11, and a negative effect (less activity) for pH values ≤5. The positive effect of the salt in the basic pH range is probably due to the acidifying effect of MgCl$_2$ mitigating the stress at high pH. Absolute differences in results between figures 2A and B are due to differences in the sensitivity of the two diagnostic methods with plate reader assays being inherently less sensitive than culture. Statistical analysis of redox activities revealed significant differences between the mean increases in activity signals obtained for different treatments. Significance (p) was less or equal than 0.05.

Effect of salt concentration

To address the dependence of the effect on the salt concentration, *L. monocytogenes* was exposed to phosphate buffer at pH3 (initial pH without salt) supplemented with increasing concentrations of MgCl$_2$ or NaCl (figure 3). Both salts affected viability, but at different concentrations. Compared with a control sample without salt, the presence of MgCl$_2$ and NaCl reduced growth in concentrations ≥150 mM and ≥400 mM, respectively. This phe-
Figure 2. Effect of pH in the absence (−) and presence (+) of 400 mM MgCl₂ on culturability (A) and redox activity (B) of *L. monocytogenes*. Cells were exposed to different pHs for 20 min (measured pH values of mixed buffer-salt solutions are indicated) followed by resuspension in neutral buffer. A) Serial dilutions of cells spotted on TSA. Pictures of representative plates are shown. B) Effect of salts and pH on redox activity. Values show the increase of WST-8 signals within 3 h. Error bars represent standard deviations from three independent experiments.
Figure 3. Effect of increasing concentrations of MgCl$_2$ and NaCl on *L. monocytogenes* at low pH. Cells were exposed for 20 min to different concentrations of MgCl$_2$ and NaCl at pH 3 (initial pH), followed by assessment of culturability (after resuspension of cells in neutral buffer, serial dilution, and spotting of aliquots on TSA) in comparison with a control without salt. pH values of mixed buffer-salt solutions are indicated. Pictures of representative plates are shown.

The phenomenon might have been caused synergistically by increasing osmolarity and the aforementioned pH.

Membrane integrity

In addition to assessing culturability and redox activity, the impact of the two salts was studied using fluorescence microscopy by staining treated cells with SYTO9 and propidium iodide (as part of the LIVE/DEAD® BacLight™ kit). A green color indicates an intact membrane, a red color indicates membrane damage. Whereas all cells appeared green at pH 7 (initial buffer pH), independent of the presence of salt, a few red cells were observed at pH 3 without salt and in the sample containing NaCl (figure 4). In the sample with an initial pH of 3 supplemented with MgCl$_2$, more than half of the cells stained red.

Testing other bacterial species

To test whether our observations held true for a wider range of bacterial species, pure cultures of *E. coli*, *S. typhimurium*, *E. faecium*, and *S. aureus* were exposed to pH 7 and acidic pHs in the absence of salt or in the presence of MgCl$_2$ and NaCl (figure 5). Due to the different tolerances of the different species towards acid stress, pH 3 was chosen for the two gram-negative species and pH 2 for the two gram-positive species. No salt effect was observed for the initial buffer pH of 7 for any of the bacteria. At acidic pHs the presence of MgCl$_2$ almost completely abolished growth of all of the species, whereas the effect of NaCl varied between species. Whereas it had no effect on the survival of gram-negative species, the impact on gram-positive species was comparable to that seen with MgCl$_2$.

Comparison of different anionic bases

To assess whether the effect is limited to the presence of phosphate, *L. monocytogenes* was
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Figure 5. Effect of exposure of E. coli, S. typhimurium, E. faecium and S. aureus to MgCl₂ and NaCl at pH7, pH 3, or pH2. Cells were exposed to 400 mM salt for 20 min each. A cell suspension without salt served as a control. pH values of mixed buffer-salt solutions are indicated. Following exposure, cells were resuspended in neutral buffer, serially diluted, and aliquots spotted on TSA. Pictures of representative plates are shown.

The effect of MgCl₂ on viability was again seen for both tests in the presence of acetic acid/acetate, and especially of lactic acid/lactate. The presence of salt resulted in both cases in a substantial drop in pH.

Effect of the salt anion

To study the effect of the salt anion, experiments at neutral and acidic pH were performed in the presence of MgSO₄. Samples without salt and supplemented with MgCl₂ and NaCl served as controls (figure 7). The NaCl concentration (800 mM) chosen was twice as high as the MgCl₂ concentration (400 mM) in order to obtain the same chloride concentration. As expected, no effect of either salt was observed at neutral pH on viability (cultivation and redox activity) of L. monocytogenes. At acidic pH both chloride salts had an impact on the two viability parameters (with MgCl₂ showing more effect than NaCl), whereas the sample containing MgSO₄ appeared comparable with the one without salt.

Discussion

Results presented in this study indicate that at low pH and in the presence of inorganic or organic bases, extracellular MgCl₂ at concentrations above 150 mM can result in an antimicrobial effect. As the salt does not affect bacterial viability under ‘normal’ conditions (i.e. near-neutral pH), the action qualifies as synergistic with acidity. The effect of MgCl₂ was substantially stronger than that exerted by other chloride salts and might, in part, be explained by enhanced acidification. The latter can be attributed to the special properties of the Mg²⁺ cation which has, among the biologically relevant cations, the smallest ionic radius and the highest charge density [11]. This in turn results in a strong interaction with the water molecules that surround the cation in two shells [12]. The polarity induced by the high charge density of the central Mg²⁺ cation renders water molecules more...
Figure 6. Simplified models to explain the roles of the cation and anion. A) Schematic diagram of how inorganic and organic bases increase the acidity of dissolved MgCl₂. Mg²⁺ cations are surrounded by 18 water molecules forming two hydration shells. Whereas aqueous MgCl₂ is a weak acid with polarized water molecules releasing protons, the presence of inorganic and organic bases enhances the release of protons resulting in increased acidification. B) Speciation analysis of MgCl₂ and MgSO₄ in the pH range between pH1 to pH7. The diagram shows the molar concentrations of free Mg²⁺ and the corresponding ionic couple.
Antimicrobial properties of MgCl₂

Figure 7. Effect of different buffers/bases at pH4 on culturability of L. monocytogenes in absence or presence of MgCl₂ (400 mM). pH values of mixed buffer-salt solutions are indicated. Cells were exposed to different buffer-salt mixtures for 20 min followed by resuspension in neutral buffer, serial dilution and spotting of aliquots on TSA. Pictures of representative plates are shown.

likely to donate a proton than in the absence of salt. Depending on the point of view, Mg²⁺ ions therefore qualify as a Lewis acid (accepting electrons, based on the Lewis acid-base theory), or hydrated Mg²⁺ can be seen as an acid (donating protons to a base, in agreement with the Brønsted-Lowry acid-base theory). Although there might be other possible explanations, the strong polarity of Mg²⁺ is likely to cause a slightly acidic pH when dissolving MgCl₂ in water.

Whereas the polarity of Mg²⁺ explains why an aqueous MgCl₂ solution is slightly acidic, the acidification is greatly enhanced in the presence of strong bases resulting in stronger hydrolysis (as schematically summarized in figure 6A). The acetate or lactate used in this study serve as examples of molecules that can increase acidity in the presence of MgCl₂, whereas no effect is seen with TRIS (figure 7). The reason for the latter can be seen in the fact that at a pH substantially lower than its pKₐ (8.3), the protonated and thus positively charged TRIS is not a base. In the cases of acetic acid (pKₐ = 4.79) and lactic acid (pKₐ = 3.86) on the other hand, the acid-base ratio at the experimental pH of 4 produces sufficient base molecules to result in acidification in the presence of MgCl₂. Acidification, in turn, shifts the acid-base equilibrium towards acidic. Only the protonated, uncharged forms of these molecules exert an antimicrobial effect, a mechanism shared among weak-acid preservatives [13]. The difference in the pKₐ values of the two acids is probably reason why the antimicrobial effect of the salt-acid mixture was substantially stronger in the presence of lactate than with acetate. The acidification of MgCl₂ in the presence of phosphate buffer on the other hand, might be better explained by the low solubility of magnesium phosphate salts. The protons left behind after precipitation result in acidification. The acidification caused by different concentrations of MgCl₂ in the presence of phosphate buffer is shown in table 1.

Apart from the role of the Mg²⁺ cation, the comparison between MgCl₂ and MgSO₄ (figure 8) raises the question how much of the effect can be attributed to the anion. Both salts were compared in the presence of phosphate buffer; when added

Table 1. Effect of different concentrations of MgCl₂ on pH when added to phosphate buffer of defined initial pH. Numbers show final pH values after addition of aqueous salt solution. The final concentration of phosphate buffer after MgCl₂ addition was 50 mM.

<table>
<thead>
<tr>
<th>initial pH</th>
<th>MgCl₂ concentration (in mM)</th>
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<th>50</th>
<th>150</th>
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to neutral phosphate buffer, acidification resulted. When added to phosphate buffer at pH3 on the other hand, acidification was only observed with MgCl₂. Differences in speciation of the two salts at low pH might be a potential explanation of this phenomenon. A computer-based analysis revealed a slightly higher concentration of free Mg²⁺ with MgCl₂ compared to MgSO₄ (figure 6B), suggesting that free Mg²⁺ might play a role. A full speciation analysis for MgCl₂ when dissolved in phosphate buffer can be found in table 2 (Appendix). Further research will, however, be necessary to investigate the differences between these salts in relation to their different biological effects.

Although the conditions applied in this study are ‘artificial’, they demonstrate that different salts have very different effects on bacterial viability and that the effects are pH-dependent. At pH conditions that are critical but sub-lethal, the additional presence of the salt can render cells more susceptible, exceeding the tolerable stress intensity. Acidic pH is one of nature’s most efficient strategies to control microbial growth. Low pH is, for example, an essential requirement for healthy skin where the pH has been reported to be, on average, around 4.7 as a result of acids that are either secreted by the human body or produced by bacteria that are part of normal skin flora [14]. Disturbance of this protective mantle is common in skin disorders such as atopic dermatitis and eczema [15]. An increase in skin pH can be associated with a general increase in skin colonization, a higher abundance of pathogens [16], and modulated virulence of pathogens [17] and their adhesion [18]. In the context of this study, it is noteworthy that MgCl₂ is the dominant salt in the Dead Sea, with a Mg²⁺ concentration of 1.89 M and Cl⁻ representing 99% of all anions [19]. The Dead Sea has been credited with healing properties for skin diseases since historic times. Scientific studies on its effect on microbes are extremely rare, although antimicrobial properties have been described for Dead Sea mud [20]. Interestingly, we could show in this study that Staphylococcus aureus (which is a common skin pathogen) was susceptible to the presence of MgCl₂ at an acidic pH. Microbiological studies in relation to skin seem appropriate for future research. Anionic bases can be expected to be present on skin in the form of skin excretion products, bacterial metabolites and cellular debris from dead keratinocytes accumulating on the surface. In contrast to the harsh acidic pH conditions (typically pH 3) chosen in this study to look for an antimicrobial effect within a short exposure time (20 min), less severe (and thus more physiologically relevant) pH conditions might be effective when applying longer exposure times. It is tempting to speculate that it might be beneficial to apply alpha hydroxy acids (AHAs) in combination with MgCl₂ for skin treatment. AHAs comprise a group of organic carboxylic compounds (lactic acid, glycolic acid, malic acid, citric acid, etc.) commonly used in cosmetics and dermatological applications [21]. Our findings might also shed new light on AlCl₃ which is a common ingredient of deodorants, its effect being attributed to the blocking...
Table 2. Molar concentrations (M) of chemical magnesium and phosphate species at different pH values. The speciation analysis is based on 400 mM of MgCl₂ dissolved in 50 mM phosphate buffer.

<table>
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<tr>
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<tr>
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</table>

of sweat glands [22] and a direct toxicity of Al³⁺ [23]. Extrapolation of the findings of this study might suggest that, in common with MgCl₂, some of the antimicrobial action might also be explained by the decrease in pH (Al³⁺ is a stronger Lewis acid than Mg²⁺) [24]. Although a stronger antimicrobial effect can be obtained with such metals, an obvious advantage of MgCl₂ over more toxic salts consists in the harmless nature of the salt as regards to human health and environmental impact.

Despite the focus on the enhancement of pH stress (which we currently see as the dominant factor for the explanation of the results obtained), other potential mechanisms of action of MgCl₂ cannot be excluded. Figure 2 suggests that acidification might not be the only factor responsible for the impact on bacterial viability. Comparing samples with and without MgCl₂, viability was affected more strongly at a final pH of 3.3 in the presence of salt than at pH 3.0 in the absence of salt. Similarly, survival was lower at pH 2.5 with salt than at pH 2 without salt. Additionally, results suggest distinct pH-independent effects of different salts as demonstrated in the direct comparison of MgCl₂ and NaCl (figure 3), where the two salts exert different effects on bacterial viability at comparable pH values. These specific salt effects which seem to add to the pH effect will need confirmation and further investigation. Factors such as osmotic pressure, transport mechanisms, and interaction of the ions with proteins and lipids have to be considered. Apart from affecting membrane permeability and membrane potential, MgCl₂ has been shown (for eukaryotic cells) to interact with a large number of exchangers and channels found in cellular membranes [7]. As regards to osmotic stress, Listeria has been reported to be extremely resistant. Liu et al. (2005), when examining the salt tolerance of different virulent and avirulent L. monocytogenes strains, found that all strains tested were resistant to saturated NaCl (corresponding to approximately 6.1 M) for at least 20 h and possibly longer, as tested by enumeration of colony-forming units [25]. This finding is in line with a later study showing that no decrease in viability was obtained when exposing Listeria to a highly concentrated NaCl solution (4.8 M) at neutral pH for three hours [5]. Osmotic stress should therefore not contribute greatly to the observations reported here.

In summary, this study demonstrates the effect that the presence of a ‘harmless’ salt can have on microbial viability. Whereas MgCl₂ does not visibly affect cells under ‘normal’ conditions, its presence can have a severe impact under critical (but yet sublethal) conditions. Although we hypothesize that the effect is largely due to a drop in pH, other factors might be involved in this antimicrobial activity. Future studies will greatly benefit from the incorporation of a skin model, given its potential implications for dermatological applications.
Disclosure

Financial support: this work was, in part, supported by the Chilean Council for Science and Technology (Project FONDECYT 1101009). Conflict of interest: none.

References