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Silicates characterization as potential bacteriocin-carriers

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ABSTRACT

Two different silicates, zeosil and expanded perlite, were characterized as potential carriers of a bacteriocin with anti-*Listeria monocytogenes* activity, produced by *Enterococcus faecium* CRL1385. Specific surface areas showed a value significantly higher for zeosil (146 m^2 g^{-1}) than for perlite (0.65 m^2 g^{-1}). Potential zeta measurements revealed that both silicates had negatively charged surfaces between pH 2 and 11, but zeosil presented zero charge near pH 2. Fourier Transform Infrared (FTIR) spectra proved that zeosil presented more silanol groups available for bacteriocin interaction than perlite. Bacteriocins present in the cell-free supernatant (CFS) were adsorbed by both silicates. Adsorption was highest from pH 4 to 8 and, regardless of exposure time (0.5 or 4 h) and silicate concentration (1 or 4% w/v) at 25 °C. Bacteriocin adsorption onto zeosil (*ca.* 99%) was higher than onto expanded perlite (*ca* 80%). However, antimicrobial activity of bacteriocins adsorbed onto perlite was higher than onto zeosil. After 2 h contact between *L. monocytogenes* 01/155 cells and each silicate plus the bacteriocin sample, the number of *Listeria* viable cells decreased close to 2 and 6 log orders for zeosil and expanded perlite, respectively.

Industrial relevance: One of the crucial problems in the use of bacteriocins as food biopreservatives is obtaining and purifying these antimicrobials. The process generally has a poor yield and is industrially expensive. Hence, alternative techniques to deliver bacteriocins may be a likely option to encourage their use as bioprotectors. Silicates, inert compounds of large surface area, are suggested in this work as peptide immobilizers so that they may later be used in food. These inorganic compounds have already been authorized as food-grade anticaking, clarifying or filtering agents. The results achieved so far with adsorption and anti-*Listeria* activity preservation of bacteriocin, once they have been immobilized onto silicates, offer a promising and simple alternative to incorporate this compound into food.

1. Introduction

Protein adsorption has been actively studied for decades because of its importance in a wide range of biomedical and industrial applications (Suzawa & Shirahama, 1991; Horbett & Brash, 1987; Norde, 1986). For example, proteins are often used in the stabilization of emulsions and foams in the food industry. In medical science there is a growing interest in the adsorption of proteins on implant materials and in the design of drug delivery systems. In biotechnology, enzymes are immobilized in bioreactors (Van der Veen, Norde, & Stuart, 2004; Nakanishi, Sakiyama, & Imamura, 2001) with several advantages, for instance as reusable heterogeneous biocatalysts, with the aim of reducing production costs by efficient recycling and process control. Particularly, the interaction of proteins with silicates found several different applications. For example, perlite and its expandable forms are used as low cost dye adsorbents, lightweight aggregates in the construction industry; rooting media and soil conditioners in horticulture, bleaching agents in the textile industry and adsorbents in the chemical industry (Topçu & Işıkdağ, 2007). However the interaction between this kind of silicates and proteins or peptides like bacteriocins has scarcely been studied.

Bacteriocins are antimicrobial, proteinaceous compounds produced by different types of bacteria (Jack, Tagg, & Ray, 1995). These cationic, hydrophobic, extracellular peptides are excreted by the producer cells, and inhibit or stop other bacterial growth, usually by adsorption to specific receptors on the external surface of sensitive bacteria, followed by metabolic, biological and morphological changes (Cleveland, Montville, Nes, & Chikindas, 2001; Daw & Falkiner, 1997). Although bacteriocins may be produced by Gram-positive and Gram-negative bacteria, those from lactic acid bacteria (LAB) are of particular interest due to their potential use in the food industry as natural and safe food

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preservatives (Gálvez, Abriouel, López, & Omar, 2007; Chen & Hoover, 2003; O'Sullivan, Ross, & Hill, 2002).

Many authors have developed different strategies for the purification of these molecules taking into account their physicochemical characteristics (Muriana & Luchansky, 1993; Yang, Johnson, & Ray, 1992) and some suggested the use of inorganic supports as an alternative (Janes, Nannapaneni, Proctor, & Johnson, 1998; Coventry, Gordon, Alexander, Hickey, & Wan, 1996; Wan, Gordon, Hickey, Mawson, & Coventry, 1996; Bower, McGuire, & Daeschel, 1995; Daeschel, McGuire, & Al-Makhlafi, 1992). However, there are few reports concerning bacteriocin–silicate interaction as an alternative to deliver these food bioprotectors (Deegan, Cotter, Hill, & Ross, 2006; Dawson, Harmon, Sotthibandhu, & Han, 2005).

Given that bacteriocins have become of great interest in food biopreservation, health care and pharmaceutical applications (Daeschel, 1993; Harlander, 1993) and that silicates like diatomaceous earths and porous silica-derived compounds have been used as food-grade anticaking, clarifying, and filtration agents (Anonymous, 1991; Baranowski, 1990), the results obtained for the characterization of two silicates and their interaction with the enterocin synthesized by *Enterococcus faecium* CRL1385 are presented in this work.

2. Materials and methods

2.1. Sorbents

Two different silicates provided by Porfenc S.R.L. (Buenos Aires, Argentina) were analyzed as potential bacteriocin carriers: i) Zeosil®175 (Rhodia, Brazil) a synthetic, highly dispersible precipitate amorphous silica; and ii) expanded perlite, obtained by quickly heating (850–900 °C) perlite, a naturally occurring siliceous rock or amorphous volcanic glass.

2.2. Sorbents characterization

2.2.1. Specific surface area

Both silicates specific surface areas were measured by N_2 adsorption at liquid nitrogen temperature (BET) using Micromeritics Flow sorb II 2300 equipment.

2.2.2. Fourier Transform Infrared (FTIR) spectroscopy

FTIR spectra of the silicate samples, dispersed in KBr wafers, were obtained in the 4000–500 cm^{-1} range using a Brucker IFS 88 spectrophotometer in the absorption mode. The samples studied were silicates in the following conditions: a) without any treatment, b) after thermal treatment in an autoclave (121 °C for 15 min), c) after thermal treatment in an autoclave (121 °C for 15 min) and exposure to a suspension of bacteriocin (2 h – 25 °C in a 4% w/v ratio), and d) a control with LAPTg sterilized culture medium in the same conditions as c).

2.2.3. Surface charge

Both silicates surface charge was determined from the electrophoretic mobilities measured at 25 °C using a Zetameter System 3.0 set at the stationary planes of a cylindrical microelectrophoresis cell. Appropriate amounts of the solids were suspended in 0.01 M KCl to maintain a constant ionic strength and left standing overnight. Suspension pH was adjusted with either 0.1 M HCl or KOH. The reported data refer to measurements after a 15 min equilibration period.

2.3. Bacterial strains and culture media

E. faecium CRL1385, a lactic acid bacterium that synthesizes bacteriocin with anti-*Listeria* activity (Audisio, Oliver, & Apella, 1999, 2001), and *Listeria monocytogenes* 01/155 (Instituto de Microbiología "Dr. Carlos Malbrán", Buenos Aires, Argentina) were used. *E. faecium* CRL1385 was routinely propagated in LAPTgm (Raibaud, Caulet, Galpin, & Mocquot, 1961) modified by (Audisio, Oliver, & Apella, 1999): meat peptone 1%; yeast extract 1%; tryptein 1%; glucose 1%; Tween 80% 0.1%, pH 6.5 at 37 °C for 12 h. *L. monocytogenes* 01/155 was cultured in brain–heart infusion broth (BHI, Britania, Buenos Aires, Argentina) at 37 °C for 12 h. Stock cultures were stored at −20 °C in BHI broth containing 10% (w/v) glycerol.

2.4. Bacteriocin solution preparation

To prepare the bacteriocin solution (BS), *E. faecium* CRL1385 was cultured in LAPTgm broth at 37 °C for 6 h. Cells were removed from the fermentation broth by centrifugation (10,000*g* for 20 min at 10 °C). Then, the cell-free supernatant (CFS) was filter-sterilized (0.45 μm pore size) and the resultant BS was used for adsorption studies and kept at 4 °C till assayed. Bacteriocin activity was determined in duplicate by the critical-dilution method (Daba, Pandian, Gosselin, Simard, Huang, & Lacroix, 1991), using *L. monocytogenes* 01/155 as the indicator culture. Briefly, *Listeria* cells were grown overnight in BHI broth to reach mid-exponential phase. Then, cells were harvested by centrifugation (5000*g* for 10 min at 25 °C), resuspended in 0.1% (w/v) peptone water and the concentration adjusted to 10^6 cfu ml^{-1}. Molten BHI agar was cooled to 45 °C and subsequently seeded with 1% (v/v) of the suspension. The inoculated medium was poured into petri dishes and dried for 30 min. BS was two-fold serially diluted in sterile distilled water and aliquots (23 μl) of each dilution applied in 5 mm wells made in the BHI agar plate. The plate was incubated at 25 °C for 20 h. The suspension titer was expressed in arbitrary units per milliliter (AU ml^{-1}) and calculated as follows: $(1000)/(V_s \times D)$, where V_s: bacteriocin volume tested (μl), D: the highest dilution that still inhibits cell growth. These determinations were performed in triplicate.

2.5. Bacteriocin adsorption on silicates

The adsorption of bacteriocin solution (BS), with an anti-*Listeria* activity titer of 11,130 UA ml^{-1}, was studied taking into account with exposure time, sorbent mass and bacteriocin solution pH.

2.5.1. Exposure time

The sorbent, zeosil or expanded perlite, and the BS at pH 4 were in contact at a constant 4% w/v concentration. Mixtures were kept at 25 °C and at different times (0, 0.5, 1, 2, and 4 h), aliquots of the supernatants were recovered by centrifugation (5000*g* for 15 min at 25 °C).

2.5.2. Bacteriocin solution pH

BS aliquots, adjusted between pH 2 and 10 with 5 M HCl or NaOH, were in contact with the sorbents at a constant 4% w/v ratio. After 2 h at 25 °C residual BS was recovered by centrifugation (5000*g* for 15 min at 25 °C). BS aliquots at the different pH and without previous exposure to the silicates were used as control.

2.5.3. Sorbent mass

BS aliquots at pH 4.0 were exposed to each sorbent in a 1, 2 and 4% w/v ratio. The mixture was kept at 25 °C for 2 h and the supernatants were recovered by centrifugation (5000*g* for 15 min at 25 °C).

In the three assays, residual bacteriocin activity in the supernatants recovered by centrifugation was determined against *L. monocytogenes* 01/155 by the critical-dilution method (Daba et al., 1991) as explained above (Section 2.4).

2.6. Inhibitory effect of bacteriocin adsorbed on silicate against *L. monocytogenes* 01/155

Adsorbed bacteriocin silica was prepared as stated previously: 10 ml BS aliquots (pH 4.0) were exposed to 0.4 g of each sorbent (4% w/v ratio); the mixture was kept at 25 °C for 2 h and the silica pellets were recovered by centrifugation (5000*g* for 15 min at 25 °C). The adsorbed bacteriocin silica was exposed to a pellet of *L. monocytogenes* 01/155, (*ca.* 1×10^8 cfu ml^{-1}) in an Erlenmeyer flask with peptone-

water solution at 25 °C without stirring in order to evaluate the anti-*Listeria* activity of adsorbed bacteriocin. Erlenmeyer flasks with 0.4 g silica exposed to 10 ml peptone water for 2 h at 25 °C instead of BS were used as control of silica activity on the pathogen. Aliquots of the supernatants of the *Listeria* suspension exposed to the silica, were taken at different times (0, 0.5, 1, 2, 4 h), decimal dilutions were made and *Listeria* viable number was determined by plate count in BHI agar. The plates were incubated at 37 °C for 24 h. Residual bacteriocin titre in the reaction medium was quantified by serial dilutions in the same aliquots used for viable cell count. Percentage of adsorbed bacteriocin activity was also calculated as: [(Initial BS activity − residual BS activity) / initial BS activity × 100].

2.7. Temperature effect on adsorbed bacteriocin stability

Once adsorbed bacteriocin perlite was prepared as explained before (Section 2.6), the resulting material was sterile dried at 50 °C for 48 h and stored in a drier at 25 °C. After this thermal treatment, anti-*Listeria* activity of the adsorbed bacteriocin was determined as follows: 10 ml of a *L. monocytogenes* 01/155 (*ca.* 10^8 cfu ml^{-1}) suspension was added to Erlenmeyer flasks with the dry mixture of perlite adsorbed bacteriocin and samples were taken to determine pathogen viability in the supernatant after 1, 2 and 4 h of exposure. In all cases, the *L. monocytogenes* growth was followed in a silica control hydrated with sterile peptone water and subjected to the same conditions as the perlite adsorbed bacteriocin.

3. Results

3.1. Sorbents characterization

The sorbents were characterized prior to analyzing their potential as bacteriocin-carriers. Surface area was significantly larger for zeosil (146 m^2 g^{-1}) than for perlite (0.65 m^2 g^{-1}). Zeta potential measurements indicated that perlite has a negatively charged surface between pH 2 and 11 with a minimum negative charge close to pH 9.5 while zeosil shows a decrease in negative charge between pH 11 and 2 with zero charge close to pH 2 (Fig. 1).

The FTIR spectra for perlite and zeosil, without any treatment and after different assays, are presented in Fig. 2. Silicates were also analyzed after thermal treatment in an autoclave to evaluate the sterilization effects since this treatment (121 °C for 15 min) would be used in the sorption process to ensure sterility during the different microbiological assays. Normal perlite presents bands at 3440 cm^{-1} and 1620 cm^{-1} (Fig. 2A-i and B-i, respectively) corresponding to the angular deformation of physisorbed water. Additionally, a well defined and sharp shoulder is observed at 3675 cm^{-1} indicating the presence of silanol groups forming hydrogen bridge bonds. When perlite is subjected to the thermal treatment explained before, the shoulder at 3675 cm^{-1} does not resolve any longer and the band corresponding to silanol groups becomes very broad and weak, indicating that the silanol groups are interacting with the water in the autoclave atmosphere (Fig. 2A-ii). The same occurs with perlite after being exposed to the culture medium and the bacteriocin (Fig. 2A-iii and iv, respectively). Maximum absorption occurs at 3467 cm^{-1} for thermally treated perlite and at 3452 cm^{-1} in the other cases. In the region of Si–O vibrational modes for perlite (Fig. 2B-i), a band at 1028 cm^{-1} and a shoulder at 1174 cm^{-1} due to Si–O antisymetric stretching and deformation respectively are observed. The presence of a weak band at 730 cm^{-1} indicates isolated Si–OH groups that are not accessible for adsorption since they do not modify their position after exposure to the culture medium or the protein. On the other hand, normal zeosil (Fig. 2C-i) presents an intense, wide band with a maximum of 3421 cm^{-1}, indicating Si–OH interaction with physisorbed water. As in the case of perlite, the angular deformation at 1631 cm^{-1} (Fig. 2D-i) confirms this situation. A weak shoulder with little resolution, characteristic of silanol groups linked by a hydrogen bridge, is also detected in this region at 3651 cm^{-1} (Fig 2C-i). The position and shape of these bands undergo very slight changes with later treatments. The high intensity adsorption band at 1092 cm^{-1}, corresponding to Si–O asymmetric stretching characteristic of silica gels, and a shoulder at 1199 cm^{-1}, are indicative of a degree of disorder in the silica network (Murgia, Farfán Torres, Gottifredi, & Sham, 2006). The shoulder observed at 960 cm^{-1} (Fig. 2D-i) was assigned to isolated Si–O groups that are not accessible for the adsorption of enterocins since its position modifies very little after thermal treatment or exposure to the BS or LAPTg medium.

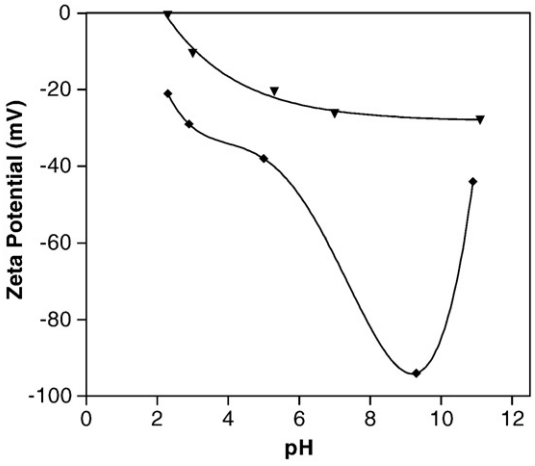

Fig. 1. Zeta potential vs. pH profiles of zeosil (▼) and perlite (♦) suspensions containing appropriate amounts of the solid suspended in 0.01 M KCl. Each point corresponds to an average of at least five readings.

3.2. Bacteriocin adsorption on silicates

The bacteriocin present in the BS of *E. faecium* CRL1385 was adsorbed onto both silicates tested, but the adsorption was higher onto zeosil than onto expanded perlite (Tables 1 and 2). Regardless of exposure time (0.5 or 4 h), the residual anti-*Listeria* activity detected in the supernatant exposed to zeosil was lower than the one measured when it was exposed to perlite (Table 1). A change in the amount of silicate in contact with the BS (1 to 4% w/v) did not modify significantly the rate of adsorption of the peptides (Table 1). The initial titer for BS was 11,130 AU ml^{-1} and after exposure to zeosil and perlite, the remaining titer was *ca* 100 AU ml^{-1} and 2000 AU ml^{-1}, respectively. This result shows that the percentage of adsorbed bacteriocin activity, calculated as the activity found on the silica after adsorption divided by the amount of activity in the initial adsorption solution [((Initial BS activity − residual BS activity) / initial BS activity) × 100], was approximately 99% for zeosil and 80% for perlite. The analysis of pH effect on BS–silicate interaction indicated that the sorption of bacteriocin was pH-dependent. It was maximum between pH 4 and 8, being more significant on zeosil than on expanded perlite, It decreased substantially at pH <4 and was zero at pH 8. In the latter case bacteriocin was denatured (Table 2). From these results, an exposure time of 2 h between each silicate and BS at a 4% w/v ratio and pH 4 were the conditions for the later sorption experiences. The pH value fixed was the one from the CFS recovered after 6 h incubation of the bacteriocin producer strain.

3.3. Inhibitory effect of bacteriocin adsorbed on silicate against *L. monocytogenes* 01/155

Sorbed bacteriocin kept its antimicrobial activity against *L. monocytogenes*, although pathogen inhibition was higher in perlite bacteriocin than in that adsorbed onto zeosil. After 4 h at 25 °C, the viable number of pathogenic cells exposed to zeosil bacteriocin decreased a

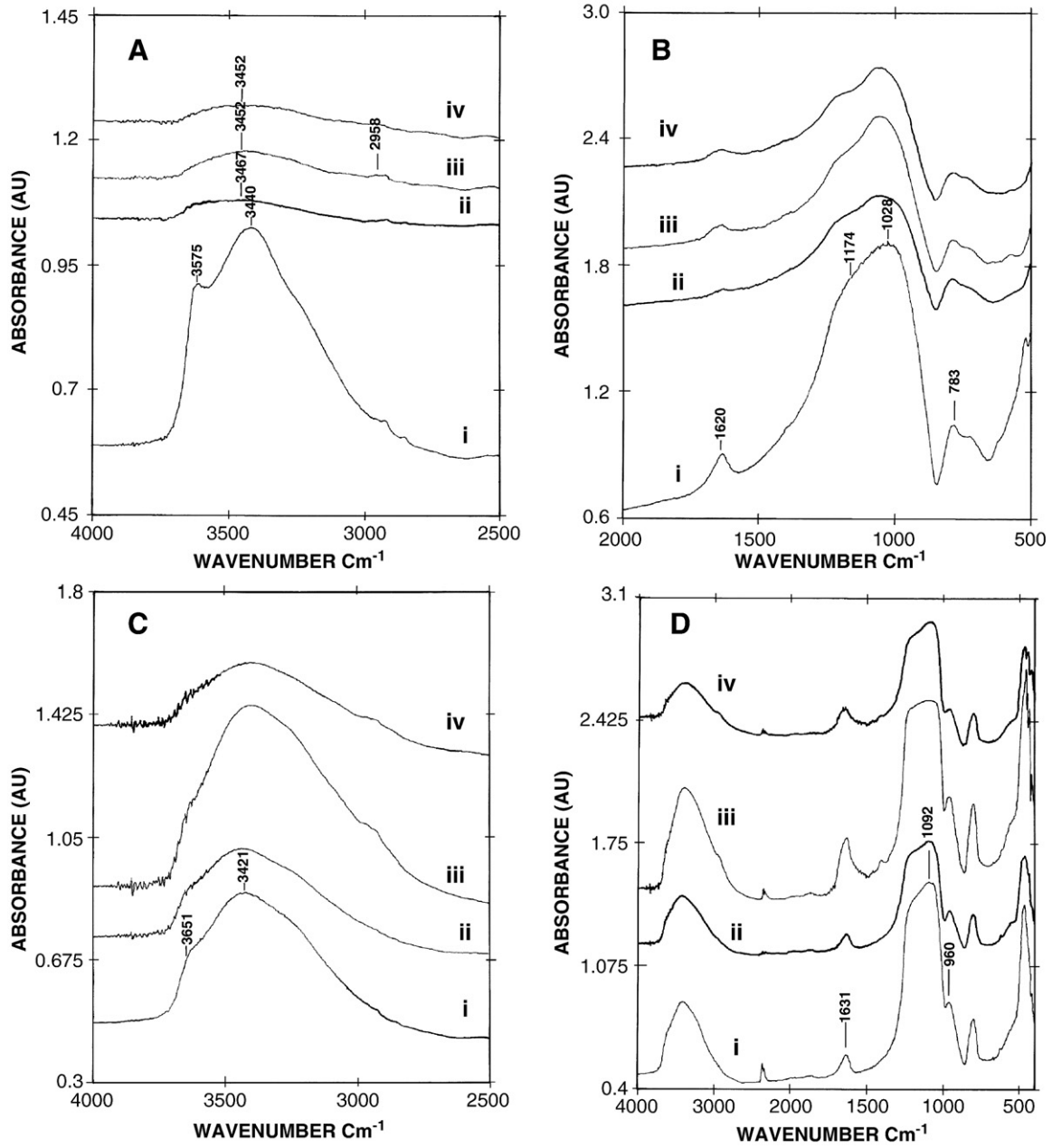

Fig. 2. FTIR spectra of perlite (A), (B) and zeosil (C), (D) without any treatment (i); after a thermal treatment in an autoclave at 121 °C for 15 min (ii), in contact with LAPTg sterilized culture medium as control (iii) and in contact with *E. faecium* CRL1385 bacterocin solution (iv).

Table 1
Residual titer and bacteriocin solution (BS) absorption percentage at different exposure times and after 2 h exposure to different amounts of zeosil and perlite at pH 4.

	UA ml^{-1}; (adsorbed bacteriocin activity (%))	
	Zeosil	Perlite
Exposure time (h)		
0	11130; (0)	11130; (0)
0.5	174; (98)	1391; (88)
1	174; (99)	1044; (81)
2	130; (99)	1044; (81)
4	130; (99)	1044; (81)
Silicate mass (% w/v)		
0	11130; (0)	11130; (0)
1	174; (98)	2783; (75)
2	43; (99)	2783; (75)
4	43; (99)	2783; (75)

L. monocytogenes 01/155 was used as indicator strain. Each point corresponds to an average of at least three independent assays.

Table 2
Effect of pH on bacteriocin solution (BS) residual antilisteria and on its adsorption efficiency activity, after 2 h exposure to zeosil or perlite at a constant 4% (w/v) ratio.

pH	UA ml^{-1}; (adsorbed bacteriocin activity (%))		
	BS[a]	Zeosil	Perlite
1.83	2787	349; (87)	697; (75)
4.00	11150	43; (99)	1397; (88)
5.40	5576	0; (100)	349; (94)
7.20	697	0; (100)	86; (88)
8.10	86	0; (100)	86; (0)
8.95	0	0; (n/d)	0; (n/d)
10.03	0	0; (n/d)	0; (n/d)

L. monocytogenes 01/155 was used as indicator strain. Each point corresponds to an average of at least three independent assays.

[a] A BS of each pH without silicate exposure was used as control of pH effect on bacteriocin antimicrobial activity.

little more than 1 order (*ca.* 6.8 log cfu ml^{-1}) with reference to the control (*ca.* 8.0 log cfu ml^{-1}), while the viable number of cells exposed to bacteriocin adsorbed onto perlite decreased more than 6 orders (Fig. 3). The activity of the bacteriocin delivered to the reaction media agrees with the viability of the pathogen observed. It was practically constant for 4 h and it was higher (260 UA ml^{-1}) for the perlite system than for zeosil system (60 UA ml^{-1}). Considering that in both cases we worked with a BS of 2780 UA ml^{-1}, the recovered activity was 2% and 10% approximately for zeosil and perlite respectively (Fig. 3).

3.4. Temperature effect on adsorbed bacteriocin stability

The characteristic thermostability of this bacteriocin was not affected by its adsorption onto perlite. Its inhibitory effect against *L. monocytogenes* 01/155 did not change after thermal treatment at 50 °C for 24 h. There were 2 orders less in the pathogen viable cell log number (Fig. 4). Perlite adsorbed bacteriocin was dried to evaluate its antimicrobial stability under similar conditions as those that silica adsorbed bacteriocin would be subjected to during its addition to dry food.

4. Discussion

Protein adsorption on solid surfaces is a complex phenomenon and involves many dynamic steps, such as the bond formation between surfaces and proteins, lateral diffusion, and conformational and/or orientational rearrangements of adsorbed proteins (Kondo & Fukuda, 1998). The ability to predict protein adsorption from mixtures thus requires taking into account the interaction between different types of proteins in the mixture and the interaction of these proteins with the surface (Gong & Szleifer, 2004). The distribution of the adsorption sites on the surface is also relevant, since more isolated sites will favour protein bonding. Electrostatic interactions are also important because most substrates and proteins are charged and the adsorbed amount is affected by various factors such as protein properties, i.e., charge, size, structure stability, amino acid composition, steric conformation and the solid substrate surface characteristics, and environmental conditions (Van der Veen et al., 2004; Haynes, & Norde, 1994).

Bacteriocin adsorption was observed in both silicates, but the higher sorption to zeosil than to expanded perlite would be related to the properties of the first silicate. It has a higher number of silanol groups observed with FTIR and a larger specific area than perlite. Silanol groups (–SiOH) are the main surface sites responsible for silica interaction with proteins and other biopolymers; therefore, alteration in their concentration can have an impact on this interaction (Mironyuk, Gunko, Turov, Zarko, & Leboda, 2001). FTIR silicate analysis leads to the conclusion that the interaction between the peptide and the silicate is higher with zeosil than with perlite. Although zeosil presents a larger number of total silanol groups, many are engaged in intraparticular interaction as a consequence of their large number and proximity or in interaction with free water. Perlite, on the other hand, has fewer silanol groups but they are more available for interaction with the medium proteins.

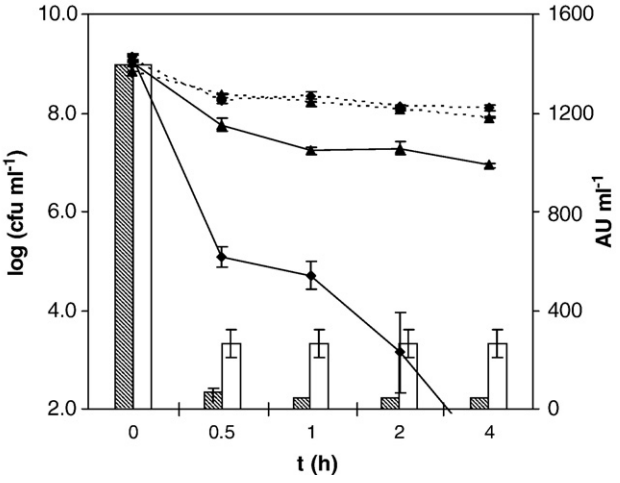

Fig. 3. *L. monocytogenes* 01/155 viability in contact with *E. faecium* CRL 1385 bacteriocins sorbed onto zeosil (▲) or perlite (♦) and antimicrobial activity recovered in the reaction medium of zeosil (marked bars) and perlite (unmarked bars). Dotted lines correspond to viability controls of *L. monocytogenes* 01/155 exposed to silicate treated with peptone-water instead of BS.

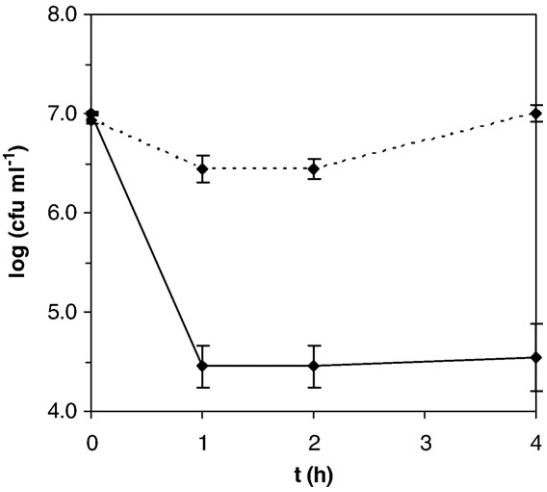

Fig. 4. *L. monocytogenes* 01/155 viability exposed to *E. faecium* CRL1385 bacteriocins sorbed onto perlite and dried at 50 °C for 48 h. Dotted lines correspond to a peptone-water adsorbed silicate control subjected to the same thermal treatment.

Since the surface of both silicates is negatively charged in the pH environment studied for BS (pH 2–10), interactions between the solid and the bacteriocin are expected to be mostly electrostatic. In this work sorption to zeosil was higher regardless of the sorbent/BS ratio and exposure time. This might indicate that we are working at saturation values.

In general, adsorption is irreversible and dependent on the concentration of the adsorbate in contact with the solid (Nakanishi, Sakiyama, & Imamura, 2001). Considering that the electrostatic interactions govern the process of adsorption, factors that affect this interaction, such as solution pH, will also affect the process of desorption. In this case, bacteriocin desorption was evidenced by the detection of antimicrobial activity of the peptide in the reaction medium without applying pH control or shaking. Nevertheless, desorption and anti-*Listeria* action detected were higher for the natural silicate. This might be explained by the lower interaction between perlite and the bacteriocin detected by FTIR. It would also indicate that a strong interaction between the peptide and the support (zeosil in this case) can lead to a bacteriocin structure modification that would negatively affect its antimicrobial activity.

5. Conclusions

Two different materials, zeosil (synthetic silicate), and expanded perlite (natural compound) were compared regarding their ability to interact with bacteriocin molecules with anti-*L. monocytogenes* activity. Both silicates were effective for bacteriocin sorption, but bacteriocin kept its antimicrobial activity better adsorbed onto expanded perlite even though the highest adsorption interaction was detected onto zeosil.

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References

Anonymous. Food additives permitted for direct addition to food for human consumption. In Code of federal regulations. Title 21, subchapter B, part 172.410, p. 45. Office of the Federal Register National Archives and Records Administration, Washington, D.C, 1991.

Audisio, M. C., Oliver, G., & Apella, M. C. (1999). Antagonistic effect of *Enterococcus faecium* J96 against human and poultry pathogenic *Salmonellae* species. *Journal of Food Protection*, *62*, 751−755.

Audisio, M. C., Oliver, G., & Apella, M. C. (2001). Effect of different complex carbon sources on the growth of *Enterococcus faecium* CRL1385 and on its bacteriocin synthesis. *International Journal of Food Microbiology*, *63*, 235−241.

Baranowski, E. S. (1990). Miscellaneous food additives. In A. L. Branen, P. M. Davidson, & S. Salminen (Eds.), *Food additives* (pp. 511−578). New York: Marcel Dekker Inc.

Bower, C. K., McGuire, J., & Daeschel, M. A. (1995). Suppression of *Listeria monocytogenes* colonization following adsorption of nisin onto silica surfaces. *Applied and Environmental Microbiology*, *61*, 992−997.

Chen, H., & Hoover, D. G. (2003). Bacteriocins and their food applications. *Comprehensive Reviews in Food Science and Food Safety*, *2*, 82−100.

Cleveland, J., Montville, T. J., Nes, I. F., & Chikindas, M. L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology*, *71*, 1−20.

Coventry, M. J., Gordon, J. B., Alexander, M., Hickey, M. W., & Wan, J. (1996). A food-grade process for isolation and partial purification of bacteriocins of lactic acid bacteria that uses diatomite calcium silicate. *Applied and Environmental Microbiology*, *62*, 1764−1769.

Daba, H., Pandian, S., Gosselin, J. F., Simard, R. E., Huang, J., & Lacroix, C. (1991). Detection and activity of a bacteriocin produced by *Leuconostoc mesenteroides*. *Applied and Environmental Microbiology*, *57*, 3450−3455.

Daeschel, M. A. (1993). Applications and interactions of bacteriocins from lactic acid bacteria in foods and beverages. In D. G. Hoover, & L. R. Steenson (Eds.), *Bacteriocins of lactic acid bacteria* (pp. 63−86). California: Academic Press.

Daeschel, M. A., McGuire, J., & Al-Makhlafi, H. (1992). Antimicrobial activity of nisin adsorbed to hydrophilic and hydrophobic silicon surfaces. *Journal of Food Protection*, *55*, 731−735.

Daw, M. A., & Falkiner, F. R. (1997). Bacteriocins: Nature, function and structure. *Micron*, *27*(6), 467−479.

Dawson, P. L., Harmon, L., Sotthibandhu, A., & Han, I. Y. (2005). Antimicrobial activity of nisin-adsorbed silica and corn starch powders. *Food Microbiology*, *22*(1), 93−99.

Deegan, L. H., Cotter, P. D., Hill, C., & Ross, P. (2006). Bacteriocins: Biological tools for bio-preservation and shelf-life extension. *International Dairy Journal*, *16*(9), 1058−1071.

Gálvez, A., Abriouel, H., López, R. L., & Omar, N. B. (2007). Bacteriocin-based strategies for food biopreservation. *International Journal of Food Microbiology*, *120*, 51−70.

Gong, P., & Szleifer, I. (2004). Competitive adsorption of model charged proteins: The effect of total charge and charge distribution. *Journal of Colloid Interface Science*, *278*, 81−90.

Harlander, S. K. (1993). Regulatory aspects of bacteriocin use. In D. G. Hoover, & L. R. Steenson (Eds.), *Bacteriocins of lactic acid bacteria* (pp. 233−247). California: Academic Press.

Haynes, C. A., & Norde, W. (1994). Globular proteins at solid/liquid interfaces. *Colloids and Surfaces B: Biointerfaces*, *2*, 517−566.

Horbett, T.A., & Brash, J.L. (1987). Proteins at Interfaces: Current Issues and Future Pospects. In T.A. Horbett, & J.L. Brash. *Proteins at interfaces: Physico-chemical and Biochemical Studies* (pp. 1–33). Washington DC: ACS Symposium Series 343.

Jack, R. W., Tagg, J. R., & Ray, B. (1995). Bacteriocins of Gram-positive bacteria. *Microbiological Reviews*, *59*, 171−200.

Janes, M. E., Nannapaneni, R., Proctor, A., & Johnson, M. G. (1998). Rice hull ash and silicic acid as adsorbents for concentration of bacteriocins. *Applied and Environmental Microbiology*, *64*(11), 4403−4409.

Kondo, A., & Fukuda, H. (1998). Effects of adsorption conditions on kinetics of protein adsorption and conformational changes at ultrafine silica particles. *Journal of Colloid Interface Science*, *198*, 34−41.

Mironyuk, I. F., Gunko, V. M., Turov, V. V., Zarko, V. I., & Leboda, R. (2001). Characterization of fumed silicas and their interaction with water and dissolved proteins. *Colloids and surfaces A*, *180*, 87−101.

Murgia, V., Farfán Torres, E. M., Gottifredi, J. C., & Sham, E. L. (2006). Sol-gel synthesis of V_2O_5–SiO_2 catalyst in the oxidative dehydrogenation of n-butane. *Applied Catalysis A: General*, *312*, 134−143.

Muriana, P. M., & Luchansky, J. B. (1993). Biochemical methods for purification of bacteriocins. In D. G. Hoover, & L. R. Steenson (Eds.), *Bacteriocins of lactic acid bacteria* (pp. 41−61). California: Academic Press Inc.

Nakanishi, K., Sakiyama, T., & Imamura, K. (2001). On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon. *Journal of Bioscience and Bioengineering*, *91*(3), 233−244.

Norde, W. (1986). Adsorption of proteins from solution at the solid–liquid interface. *Advances in Colloid and Interface Science*, *25*, 267−340.

O'Sullivan, L. O., Ross, R. P., & Hill, C. (2002). Potencial of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochimie*, *84*, 593−604.

Raibaud, P., Caulet, M., Galpin, J., & Mocquot, G. (1961). Studies on the bacterial flora of the tract alimentary of pigs. II. Streptococci; selective enumeration and differentiation of the dominant groups. *Applied Bacteriology*, *24*, 285−291.

Suzawa, T., & Shirahama, H. (1991). Adsorption of plasma proteins onto polymer lattices. *Advances in Colloid and Interface Science*, *35*, 139−172.

Topçu, B., & Işıkdağ, B. (2007). Manufacture of high heat conductivity resistant clay bricks containing perlite. *Building and Environment*, *42*(10), 3540−3546.

Van der Veen, M., Norde, W., & Stuart, M. C. (2004). Electrostatic interactions in protein adsorption probed by comparing lysozyme and succinylated lysozyme. *Colloids and Surfaces B: Biointerfaces*, *35*, 33−40.

Wan, J., Gordon, J., Hickey, M. W., Mawson, R. F., & Coventry, M. J. (1996). Adsorption of bacteriocins by ingestible silica compounds. *Journal of Applied Bacteriology*, *81*(2), 167−173.

Yang, R., Johnson, M. C., & Ray, B. (1992). Novel method to extract large amounts of bacteriocins from lactic acid bacteria. *Applied and Environmental Microbiology*, *58*(10), 3355−3359.