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Antibacterial activity of bovine milk lactoferrin on the emerging foodborne pathogen *Cronobacter sakazakii*: effect of media and heat treatment

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ABSTRACT

Cronobacter sakazakii is a pathogen transmitted by food, with high osmotic resistance and tolerance to desiccation, which affects mainly to newborns, infants and immunocompromised adults. *C. sakazakii* infection in infants has been associated with consumption of powdered milk. The purpose of this study was to evaluate the antibacterial activity of native and iron-saturated bovine lactoferrin (bLF) (from 0.5 to 5 mg/ml) on non-desiccated and desiccated *C. sakazakii* (10^4 CFU/ml) in different media (phosphate buffer, bovine skim milk and whey). In general, native bLF was the only effective form that inhibited growth of *C. sakazakii* in all media, its activity increasing with concentration and time of incubation. These results suggest that the antibacterial effect of bLF on *C. sakazakii* is mainly due to iron sequestration. However, iron-saturated bLF showed some effect by reducing the viability of *C. sakazakii* in whey. There has not been observed an increased sensitivity of desiccated bacteria to native bLF in phosphate buffer. However, although the antibacterial activity of native bLF against non-desiccated *C. sakazakii* was drastically reduced in milk or whey compared to phosphate buffer, there was a certain activity when it was assayed against desiccated cells in those media. The effect of some heat treatments on the antibacterial activity of native bLF was evaluated and only those of 72℃ for 15 s, 85℃ for 15 s, and 63℃ for 30 min maintained its whole activity.

Keywords: bovine milk lactoferrin, *Cronobacter sakazakii,* antibacterial activity, heat treatment, UHT milk, whey

1. Introduction

Cronobacter sakazakii is an emerging pathogen transmitted by food that has been associated with meningitis (Burdette & Santos, 2000), sepsis (Simmons, Gelfand, Haas, Metts, & Feruson, 1989), bacteremia (Noriega, Kotloft, Martin, & Schwalb, 1990) and necrotizing enterocolitis (Van Acker et al., 2001), mainly affecting to newborns, infants, and immunocompromised adults (Lai, 2001). Recently, Iversen et al. (2008) reclassified *Enterobacter sakazakii* as a new genus, *Cronobacter*, in which fives species were included: *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii* and *C. dublinensis*. Although the outbreaks caused by this pathogen are scarce, infections with *C. sakazakii* are often accompanied by a high rate of mortality that can reach 80% (Lehner & Stephan, 2004; Kim & Beuchat, 2005). *C. sakazakii* is a Gram-negative, motile, non-spore forming, ubiquitous, facultative anaerobic bacteria, belonging to the family *Enterobacteriaceae*. It can grow over a wide temperature range (6-47ºC) and is inactivated at 70ºC. The consumption of cont aminated powdered infant formula (PIF) has been mainly associated with the majority of outbreaks caused by *C. sakazakii* (Burdette & Santos, 2000; Lai, 2001; Van Acker et al., 2001). The Codex Alimentarius Commission (CAC) of the United Nations provides regulations relevant to PIF, such as that *Cronobacter* spp. should be absent in 30 samples of 10 g in finished PIF products (CAC, 2008). The European Union officially introduced similar microbiological standards (European Commission, 2007).

C. sakazakii is usually inactivated during pasteurization of PIF (Nazarowec-White & Farber, 1997a). Therefore, the presence of this bacterium in milk can be caused by post-processing environmental contamination, addition of contaminated ingredients (Nazarowec-White & Farber, 1997b) or colonization by *C. sakazakii* of utensils used in milk preparation. It has been shown that *C. sakazakii* have a remarkable resistance in dry media for periods at least of two years (Caubilla-Barron & Forsythe, 2007). This feature represents a competitive advantage, facilitating their prevalence in products with low water content (Edelson-Mammel, Porteus, & Buchanam, 2005). *Cronobacter* spp. may accumulate solutes such as trehalose which protects the microorganism against osmotic stress by stabilizing its membrane (Breeuwer, Lardeau, Peterz, &

Joosten, 2003). Heat treatment of water at ≥70℃ for reconstitution of PIF has been recommended by FAO and WHO (2004). However, this treatment may adversely affect the sensory quality and nutritional value of this essential food for baby development. The heat treatment necessary to reduce the number of *Cronobacter* cells in milk and to avoid its proliferation could be decreased by combining it with antimicrobial compounds. According to WHO recommendations, great interest has recently grown in using natural antimicrobials, such as lactoferrin (LF), to avoid proliferation of *C. sakazakii* in infant formula. From a regulatory point of view, bovine lactoferrin (bLF) is considered safe under the proposed uses and levels in a variety of foods for nutritional applications (EFSA, 2012a,b), i.e., infant and follow-on formulas, dietary food, dairy products, yoghurts, and chewing gums (European Commission, 2012a,b).

Lactoferrin is a glycoprotein of the transferrin family present in the majority of external secretions and mucosal surfaces, milk being its main source. Lactoferrin binds two atoms of iron and due to this capacity several functions have been attributed to it, such as antibacterial, antioxidant, antitumoral and immunomodulatory (Sánchez, Calvo, & Brock, 1992a).

Almost all bacteria require iron for their growth; therefore LF devoid of iron is capable of preventing its utilization by some bacteria (Orsi, 2004). A large number of studies have demonstrated the bacteriostatic and bactericidal effect of LF, against a wide range of Gram-positive and Gram-negative bacteria (Farnaud & Evans, 2003). However, other mechanisms besides iron holding can be involved in the antibacterial activity of LF, such as blocking microbial metabolism of carbohydrates or destabilizing the bacterial cell wall (Sánchez, Calvo, & Brock, 1992a).

The aim of this study was to evaluate the antibacterial activity of bLF on the emerging foodborne pathogen *C. sakazakii* and the influence of different factors. Thus, iron saturation and concentration of bLF, desiccation of bacterial cells, media, incubation time and heat treatment have been evaluated. The effect of heat treatment is especially important since denaturation of bioactive whey proteins may result in loss of their biological functions.

2. Materials and methods

2.1. Culture of C. sakazakii and preparation of desiccated cells

A freeze-dried culture of *C. sakazakii* CECT 858, equivalent to strain ATCC 29544, was supplied by the Spanish Type Culture Collection (CECT, Valencia, Spain). After reviving freeze-dried culture, it was stored at -70℃ in sterile cryopreservation vials. Working cultures were obtained by transferring a porous bead of stock culture into 10 ml of Trypticase soy broth (TSB), incubating at 37℃ for 24 h and transferring a loop to Trypticase soy agar (TSA). After 24 h at 37℃, an isolated colony was transferred to 10 ml of TSB and was incubated at 37℃ for 24 h.

Desiccated cells of *C. sakazakii* were prepared as described by Al-Nabulsi et al. (2009), dispensing a volume of 1 ml of freshly prepared suspension from a single colony of *C. sakazakii* in 50 µl portions on a sterile Petri dish. The plate was placed at 40℃ in an incubator to be air-dried. After 2 h, the plate was placed in a desiccator at room temperature for 4 d, and afterwards, 2 ml of 0.2% (w/v) peptone water were added to the plate to collect desiccated cells, mixed with 8 ml of 0.2% peptone water and serial decimal dilutions in 1% peptone water were used to yield a suspension of 10^4 CFU/ml for antibacterial activity assays.

2.2. Preparation of native bLF solutions

Native bLF was kindly provided by Tatua Nutritionals Company (Morrinsville, New Zealand) and had an iron-saturation below 10%. The purity of bLF was checked by SDS-PAGE, which showed a single band corresponding to a protein of about 80 KDa and purity higher than 90%. The stock solution of bLF was prepared from the native protein in ultrapure water at 20 mg/ml and sterilized through a low-binding protein 0.22 µm filter. After filtration, the absorbance was measured at 280 nm and the concentration of bLF determined by considering a molar extinction coefficient ($E^{1\%}$) of 1.27 ml/cm/g. The final concentration of bLF solutions was adjusted to 1, 2, 5 and 10 mg/ml.

2.3. Preparation of iron-saturated bLF solutions

Native bLF was saturated with iron by adding ferrinitrilotriacetate (FeNTA) solution as described previously (Ismail & Brock, 1993). Afterwards, bLF was subjected to Sephadex G-25 chromatography to remove unbound iron. The iron-saturated bLF solution was filtered through 0.22 µm and the concentration was determined, considering a $E^{1\%}$ of 1.51 ml/cm/g. The concentration of solutions was adjusted to 1, 2, 5 and 10 mg/ml.

2.4. Effect of heat treatment on bLF

To study the effect of different heat treatments on the antibacterial activity of bLF against non-desiccated *C. sakazakii*, native or iron-saturated bLF were dissolved at a concentration of 5 mg/ml in phosphate buffer solution, composed by 15 mM monopotassium phosphate, 8 mM dibasic sodium phosphate, 14 mM NaCl and 2 mM KCl, pH 7.4 (PBS). A volume of 650 µl of bLF solutions was subjected to different heat treatments in sterile glass vials of 12.0 mm outer diameter and 11.6 mm inner diameter. Treatments were performed in a water bath with agitation and temperature controlled with an accuracy of ± 0.1℃. The vials were removed at several times and immediately immersed into an ice-water bath. Heat treatments performed were: 63℃ for 30 min, low-temperature long-time pasteurization (LTLT); 72℃ for 15 s, high-temperature short-time pasteurization (HTST); 72℃ for 15 min, that was chosen as an intermediate treatment between HTST and high pasteurization; 85°C for 15 s and 85℃ for 10 min, treatments used normally in the manufacture of some dairy products. Samples of heat treated bLF were subjected to electrophoresis and radial immunodiffusion to evaluate the degree of protein denaturation.

2.5. SDS-PAGE electrophoresis and radial immunodiffusion

Heat treated bLF was analyzed by SDS-PAGE. A volume of 50 µl of the treated samples was added to 40 µl of 10 mM Tris 1 mM EDTA, pH 8, and 10 µl of 25% SDS and treated at 100℃ for 5 min. Afterwards, 1 µl of each sample was applied to a 7.5 % polyacrylamide gel and electrophoresis developed in a Phast System equipment (Pharmacia Biotech, Uppsala Sweden). After the electrophoresis, the gels were stained with Coomassie blue type R.

The immunochemical reactivity of heat-treated bLF was determined by radial immunodifusion, as described previously (Sánchez et al. 1992b), by using 0.4, 0.2, 0.1 and 0.05 mg/ml bLF standards. After diffusion for 72 h, the gel was washed with saline buffer, stained with Coomassie blue type G, destained and diameters of radial precipitate measured.

2.6. Antibacterial activity assay

The media used to evaluate the antibacterial activity of native and iron-saturated bLF were: commercial ultra-high temperature (UHT) bovine skim milk, bovine whey obtained from UHT milk by ultrafiltration with 100,000 MWCO hollow fiber, and PBS. Whey and PBS were filtered through 0.22 µm.

Non-desiccated or desiccated *C. sakazakii* were diluted in 1% peptone water to achieve 10^4 CFU/ml. A volume of 100 µl of those suspensions was added to each well of a microtiter plate with 100 µl of UHT skim milk, whey or PBS containing native or iron-saturated bLF at a final concentration of 0.5, 1, 2.5 or 5 mg/ml. The final concentration of bacteria in the well was approximately 10^4 CFU/ml. Control samples consisted of media without bLF.

Heat treated bLF was assayed at 2.5 mg/ml in PBS against non-desiccated bacteria (10^4 CFU/ml). In this assay, non-treated native bLF at 2.5 mg/ml was included as reference.

The plates were incubated at 37ºC for 4 and 8 h. The number of viable cells was determined by serially diluting the content of each well in 1% peptone water and plating on TSA plates which were incubated at 37ºC for 24 h. Each well was seeded by duplicate.

2.7. Statistical analysis

Experiments were performed three times using freshly prepared samples. Mean and standard deviations were calculated from all the data obtained in the experiments performed. Data were statistically evaluated by t test and ANOVA according to Duncan test using the SPSS 19.0 package for Windows.

3. Results

3.1. Activity of native and iron-saturated bLF against non-desiccated and desiccated C. sakazakii in PBS

The results showed that native bLF in PBS exerted antibacterial activity against non-desiccated *C. sakazakii*, from 0.5 to 5 mg/ml, being significantly different from the control at concentrations above 1 mg/ml at 4 and 8 h (Fig. 1A). This antibacterial activity increased with concentration and time of incubation, and after 8 h, the 5 mg/ml bLF solution reduced the bacterial counts 7.98 log cycles, respect to the control. Iron-saturated bLF did not reduce significantly the growth of non-desiccated *C. sakazakii* at any concentration and incubation period (data not shown).

The results obtained against desiccated *C. sakazakii* (Fig. 1B) showed a significant inhibitory effect of native bLF, except for the concentration of 0.5 mg/ml, at 4 and 8 h. In general, the inhibitory effect of native bLF on desiccated cells appeared later respect to non-desiccated bacteria. The antibacterial effect of native bLF at 4 h was lower on desiccated bacteria than on non-desiccated bacteria for the same concentrations of protein. However, native bLF at 5 mg/ml produced practically the same reduction on the growth of desiccated *C. sakazakii* at 8 h, than on non-desiccated cells. The iron-saturated bLF did not show any antibacterial activity on desiccated bacteria (data not shown).

3.2. Activity of native and iron-saturated bLF against non-desiccated and desiccated C. sakazakii in bovine skim milk and whey

The antibacterial activity of native bLF against *C. sakazakii* was drastically reduced when the protein was assayed in bovine milk or whey, compared with PBS (Table 1). However, certain activity was observed for some concentrations of native bLF, being significant at 1, 2.5 and 5 mg/ml on desiccated *C. sakazakii* cells in whey after 4 h and at 5 mg/ml after 8 h, being the highest reduction of 2.96 cycles. In milk the only significant activity was found for native bLF at 5 mg/ml at 4 and 8 h on desiccated cells. The inhibitory activity of native bLF on non-desiccated cells was lower than one logarithmic cycle and only observed for 5 mg/ml.

Furthermore, significant activity was found for iron-saturated bLF at 5 mg/ml on desiccated cells in whey at 4 and 8 h of incubation, with reductions of 1.49 and 1.86 cycles, respectively (data not shown).

3.3. Effect of heat-treatment on the activity of native and iron-saturated LF against non-desiccated C. sakazakii in PBS

Treatments of 72℃ for 15 s, 85℃ for 15 s and 63℃ for 30 min did not affect the antimicrobial activity of native bLF (Table 2). However, native bLF treated at 72℃ for 15 min and at 85℃ for 10 min, showed lowe r antibacterial activity than that observed for bLF without treatment. Iron-saturated bLF did not show any antibacterial activity.

Samples of heat treated bLF in PBS were subjected to SDS-PAGE to evaluate their aggregation (Fig. 2). Some LF aggregates were observed in the stacking gel, especially for samples treated at 72°C for 15 min, 85℃ for 10 min and 85℃ for 15 s. This can be due to the establish ment of interactions between bLF molecules under heating, consequently losing its antibacterial activity.

The estimated concentrations of heat treated bLF determined by its immunoreactivity respect to the non-treated bLF are shown in Table 3. The treatments that affected bLF immunoreactivity in higher extent were 85℃ for 10 min and 72℃ for 15 min, decreasing it to 4 and 10% , respectively. After treatment of bLF at 63℃ for 30 min its immunoreact ivity decreased to 38%, although its antibacterial activity was still high as shown in Table 2. After treatment at 85℃ for 15 s the immunoreactivity was of 66% and at 72℃ for 15 s of 100% with respect to non-treated bLF.

4. Discussion

The antibacterial activity of LF has been largely demonstrated against a wide range of Gram-positive and Gram-negative bacteria (Jenssen & Hancock, 2009). During the last years some bacteria have appeared as emerging pathogens, as is the case of *C. sakazakii* (Iversen et al., 2008), and therefore, there is little knowledge of LF activity against them. Natural antimicrobials have been proposed as food preservatives in the last years, by combining them with non-thermal treatments or lower treatments, in order to maintain the nutritional and organoleptic characteristics of food (Masschalck, Van Houdt, & Michiels, 2001; Del Olmo, Calzada, & Nuñez, 2012).

Although the most recognized mechanism for LF antibacterial activity is iron sequestration, it has been shown that iron-saturated LF is also active on some

bacteria. This activity is related to its capacity to interact with the bacterial membrane, subsequently producing its destabilization, altering the bacterial metabolic processes and finally causing their death (Sánchez, Calvo & Brock, 1992a). In the present work only native bLF has been proved as inhibitory for *C. sakazakii*, therefore being iron sequestration the most probable mechanism of that activity. However, a certain inhibitory effect of iron-saturated bLF was observed on desiccated *C. sakazakii* cells in bovine whey. This could be due to changes in the bacterial membrane produced by desiccation and/or to the contribution of some whey component to the inhibitory mechanism.

C. sakazakii has been reported to be highly persistent in formulas with low water activity for long periods (Gurtler & Beuchat, 2007) and others indicated that capsulated strains of *C. sakazakii* were still recoverable from dry infant formula after two and a half years (Caubilla-Barron & Forsythe, 2007). Those studies carried out on desiccated bacteria intended to reproduce the conditions that might occur in PIF contaminated with *C. sakazakii* prior to transformation into powder. In an earlier study, Al-Nabulsi et al. (2009) reported that desiccation enhanced the sensitivity of *Cronobacter* spp. to the LF inhibitory activity, though they also found that nisin was less active on those desiccated cells. In contrast, the results obtained in this study did not show higher sensitivity of desiccated *C. sakazakii* to the effect of native bLF compared with non-desiccated cells. On the contrary, the desiccated cells reacted later to the activity of bLF and were inhibited at similar levels to the non-desiccated cells only at the highest concentration of bLF and after 8 h. This could be explained by different mechanisms reported for *C. sakazakii* to tolerate desiccation, such as synthesis of high levels of trehalose or glycine betaine (Breeuwer, Lardeau, Peter & Joosten, 2003), or production of exopolysaccharides (Jung, Choi, & Lee, 2013).

The composition of media limits the activity of antimicrobials, specially when those are complex food matrices. This has been shown in studies carried out in meat (Venkitanarayanan, Zhao & Doyle, 1999; Del Olmo, Morales, & Nuñez, 2009) or carrot juice (Chantaysakorn & Richter, 2000) in which the reduction of antibacterial activity of LF or its hydrolysates was attributed to the presence of high levels of divalent cations (Branen & Davidson, 2000; Al-Nabulsi et al.,

2009; Del Olmo, Morales & Nuñez, 2009). In previous studies we evaluated the activity of native bLF (Conesa et al, 2010), human recombinant lactoferrin from *Aspergillus awamori* (Conesa et al., 2008) and from rice (Conesa et al., 2009) on *Listeria monocytogenes* and *Escherichia coli* 0157: H7 in skim milk and whey. We found that skim milk and whey acted by protecting the bacteria reducing LF antibacterial activity. In the present work, we have also observed that bLF antibacterial activity against *C. sakazakii* is low when it was evaluated in bovine skimmed milk or whey. However, there was some activity of native and even iron-saturated bLF when were assayed on desiccated cells in milk or whey. Therefore, these results show that it is worthwhile investigating the procedures to improve LF antibacterial activity in milk and whey by modifying or eliminating some interfering components.

Considering that bLF is being used as a bioactive ingredient in some processed foods, it is essential to study the effect of heat treatment on its antibacterial activity. In the present study we found that heat treatments of 72℃ for 15 s, 85℃ for 15 s and 63℃ for 30 min maintained the whole antibacterial activity of native bLF against *C. sakazakii.* Lactoferrin subjected to the highest treatments, 72℃ for 15 min and 85℃ for 10 min, diminished in a great extent its activity, though still having some effect. The results we previously obtained of the activity of heat treated bLF and human recombinant LF from rice and fungus, on three different bacteria: *Escherichia coli* O157:H7, *Salmonella* Enteritidis and *Listeria monocytogenes* were quite coincident with those obtained in the present work, as the only heat treatment that counteracted almost completely the antibacterial activity of LF was 85℃ for 10 min (Conesa et al., 2008; Conesa et al., 2009; Conesa et al., 2010). Therefore, we can confirm that native bLF is very resistant to the most common pasteurization treatments, maintaining its antibacterial activity against *C. sakazakii*.

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FIGURE AND TABLE CAPTIONS

Figure 1. Effect of the concentration of native bLF on the growth of non-desiccated (A) and desiccated (B) *C. sakazakii* in PBS after 4 and 8 h of incubation at 37℃. Each value represents the mean ± standard deviation of nine replicates from three independent experiments. *Significant differences for $p<0.05$ with respect to the control.

Figure 2. SDS-PAGE of heat-treated native bLF. Lanes contain the following samples:1 and 7, non-treated bLF (5 mg/ml); 2, bLF treated at 63℃ for 30 min; 3, bLF treated at 72℃ for 15 s; 4, bLF treated at 72℃ for 15 min; 5, bLF treated at 85℃ for 10 min; 6, bLF treated at 85℃ for 15 s . Molecular weight markers: β-galactosidase (116 kDa), transferrin (76 kDa), glutamate deshidrogenase (53 kDa).

Table 1. Effect of the concentration of native bLF on the growth of non-desiccated and desiccated *C. sakazakii* in bovine whey and skimmed milk after 4 and 8 h of incubation at 37℃. Each value represe nts the mean ± standard deviation of nine replicates from three independent experiments. *Significant differences for $p<0.05$ with respect to the control.

Table 2. Activity of heat-treated native and iron-saturated bLF (final concentration of 2.5 mg/ml) on the growth of non-dessicated *C. sakazakii* in PBS at 4 and 8 h of incubation at 37℃. Values repr esent the mean ± standard deviation of data from three independent experiments and three replicates at each experiment. Significant differences for *$p<0.01$ and **$p<0.001$ with respect to the control. [a]n.d: not detected.

Table 3. Effect of heat treatment on the concentration of native bLF determined by radial immunodifusion using polyclonal specific antibodies. Immunoreactivity is expressed as the relative concentration respect to the non-treated bLF.

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Table 1.

Bovine whey	**Non-desiccated *C. sakazakii* (log CFU/ml)**		**Desiccated *C. sakazakii* (log CFU/ml)**	
	4 hours	**8 hours**	**4 hours**	**8 hours**
Control	5.91 ± 0.26	8.30 ± 0.06	6.05 ± 0.07	8.17 ± 0.31
LF 0.5 mg/ml	5.87 ± 0.25	8.14 ± 0.09	6.01 ± 0.40	7.99 ± 0.40
LF 1 mg/ml	5.75 ± 0.32	8.03 ± 0.07	6.01 ± 0.07	8.03 ± 0.32
LF 2.5 mg/ml	5.71 ± 0.22	7.97 ± 0.09	5.91 ± 0.12	7.90 ± 0.37
LF 5 mg/ml	5.55 ± 0.34*	7.70 ± 0.15	5.95 ± 0.10	7.91 ± 0.37
Bovine milk	**Non-desiccated *C. sakazakii* (log CFU/ml)**		**Desiccated *C. sakazakii* (log CFU/ml)**	
	4 hours	**8 hours**	**4 hours**	**8 hours**
Control	6.00 ± 0.09	8.20 ± 0.09	6.19 ± 0.05	8.32 ± 0.14
LF 0.5 mg/ml	5.92 ± 0.09*	8.10 ± 0.06	5.32 ± 0.09	8.08 ± 0.07
LF 1 mg/ml	5.78 ± 0.13*	8.00 ± 0.37	5.88 ± 0.11	7.81 ± 0.21
LF 2.5 mg/ml	5.80 ± 0.09*	7.90 ± 0.32	5.74 ± 0.21	7.57 ± 0.43
LF 5 mg/ml	5.82 ± 0.06*	7.82 ± 0.09*	4.62 ± 1.37*	6.20 ± 2. 67*

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Table 2.

	Native lactoferrin (log CFU/ml)		Iron-saturated lactoferrin (log CFU/ml)	
	4 hours	**8 hours**	**4 hours**	**8 hours**
Control	5.61 ± 0.31	7.75 ± 0.14	6.10 ± 0.11	8.61 ± 0.13
Non-treated LF	nd[a]	nd	6.04 ± 0.13	8.47 ± 0.14
LF 63℃ 30 min	nd	nd	5.94 ± 0.18	8.46 ± 0.09
LF 72℃ 15 s	nd	nd	5.98 ± 0.19	8.44 ± 0.05
LF 85℃ 1 5 s	nd	nd	5.98 ± 0.17	8.44 ± 0.04
LF 72℃ 15 min	4.83 ± 1.49*	4.12 ± 3.48**	5.99 ± 0.14	8.12 ± 0.23
LF 85℃ 10 min	4.32 ± 1.93*	3.56 ± 3.57**	5.99 ± 0.17	8.45 ± 0.05

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Table 3.

Heat treatment	% Immunoreactivity
Non-treated LF	100
LF 63℃ 30 min	38
LF 72℃ 15 s	100
LF 85℃ 15 s	66
LF 72℃ 15 min	10
LF 85℃ 10 min	4

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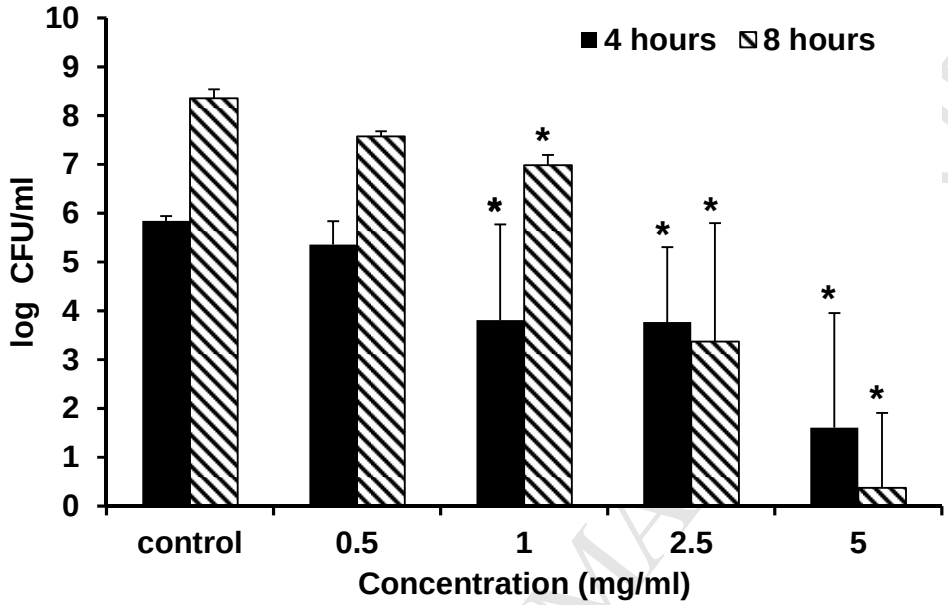
Figure 1.

A



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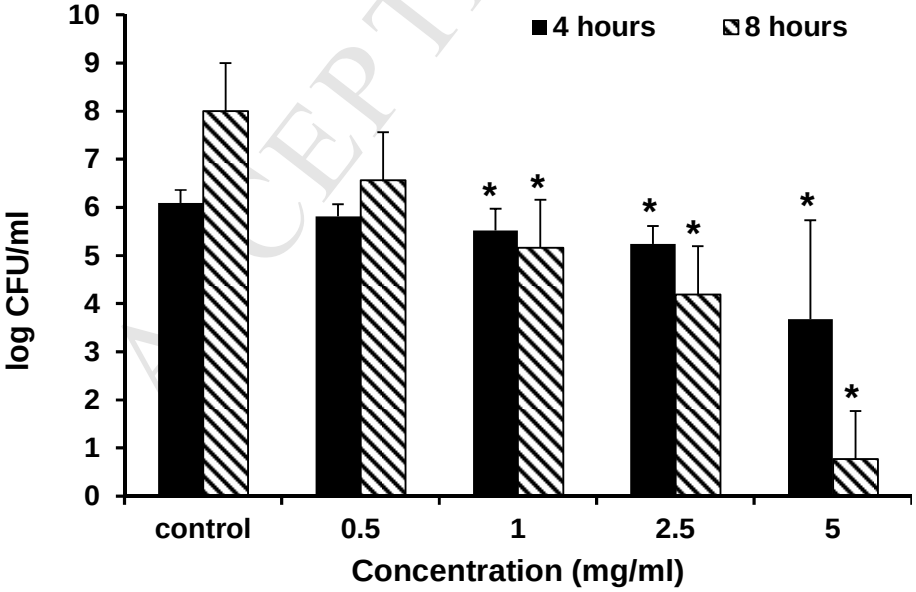

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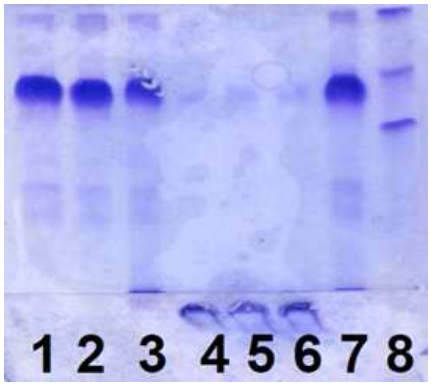
Figure 2



53 kDa

76

116

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Highlights

- Native bovine lactoferrin at 5 mg/ml reduces 8 log cycles *C. sakazakii* growth.
- Milk and whey diminish lactoferrin antibacterial activity against *C. sakazakii.*
- Pasteurization maintains lactoferrin antibacterial activity against *C. sakazakii.*

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