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Inhibitory activity of surfactin, produced by different *Bacillus subtilis* subsp. *subtilis* strains, against *Listeria monocytogenes* sensitive and bacteriocin-resistant strains

Daniela C. Sabaté, M. Carina Audisio*

Instituto de Investigaciones para la Industria Química (INIQUI-CONICET), Universidad Nacional de Salta, Argentina

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ABSTRACT

Three surfactin-producing *Bacillus subtilis* strains, C4, M1 and G2III, previously isolated from honey and intestines from the *Apis mellifera* L. bee, were phylogenetically characterized at sub-species level as *B. subtilis* subsp. *subtilis* using *gyrA* gene sequencing. The antagonistic effect of surfactin was studied against seven different *Listeria monocytogenes* strains, 6 of which were resistant to bacteriocins. Surfactin showed anti-*Listeria* activity against all 7 strains and a dose of 0.125 mg/mL of surfactin was enough to inhibit this pathogen. Surfactin sintetized by *B. subtilis* subsp. *subtilis* C4 inhibited the pathogen in lower concentrations, 0.125 mg/mL, followed by G2III and M1 with 0.25 and 1 mg/mL, respectively. In particular, a dose of 0.125 mg/mL reduced the viability of *L. monocytogenes* 99/287 RB6, a bacteriocin-resistant strain, to 5 log orders. Surfactin assayed maintained anti-*Listeria* activity within a pH range of between 2 and 10, after heat treatment (boiling for 10 min and autoclaving at 121 °C for 15 min) and after treatment with proteolytic enzymes. These results suggest that surfactin can be used as a new tool for prevention and the control of *L. monocytogenes* in different environments, for example, in the food industry.

1. Introduction

Bacillus species are ubiquitous, endospore-forming, Gram-positive bacteria that are of great economic importance due to their ability to produce a wide range of bioactive peptides. The cells are widely used in industrial processes for enzyme production (Manachini et al. 1987b; Manachini et al. 1988), especially proteases (Manachini et al. 1987a), in large-scale industrial fermentations (Fiechter 1992; Zukowski 1992) and as plant growth-promoting microorganisms (Bai et al. 2002; Joo et al. 2004). Their metabolites show antagonistic properties, so they are used against different pathogens (Czaczyk et al. 2000; Mannanov 2001; Al-Ajlani et al. 2007; Ongena and Jacques 2008). These bacteria are also employed as constituents of *probiotic supplements* for animals and humans (Cutting 2011). *Bacillus subtilis* is one of the most important species of this genus because of the synthesis of a wide range of metabolites with diverse properties (Huang et al. 2007; Etchegaray et al. 2008; Ongena and Jacques 2008; Velmurugan et al. 2009). Among these metabolites the most important compounds are the cyclic lipopeptides surfactin, iturin and fengycin, which are used in biotechnological and pharmaceutical processes due to their surface and antimicrobial properties. Surfactin in particular presents antagonistic activity against bacteria, whereas iturin and fengycin show important antifungal spectrums (Ongena and Jacques 2008; Raaijmakers et al. 2010). However, the inhibitory effect of surfactin against *Listeria* spp. has hardly been studied (de Araujo et al. 2011).

Listeria monocytogenes is a Gram-positive pathogen of humans and animals, which causes listeriosis (McLauchlin et al. 2004). In humans it is mainly transmitted by food and it is especially harmful to immunodeficient patients, elderly people, children and pregnant women (Jurado et al. 1993; McLauchlin et al. 2004; Gandhi and Chikindas 2007). Nisin, is a biological food preservative used to control *Listeria* in food (Gandhi and Chikindas 2007). However, resistant *Listeria* strains have been reported, making the search for alternative drugs or natural compounds, such as bacterial metabolites, necessary (Ming and Daeschel 1993; Holzapfel et al. 1995; Vadyvaloo et al. 2004).

In a previous study, *B. subtilis* strains were isolated in our laboratory from different honey samples and from the gut of *Apis mellifera* L. and three were selected because of their surfactin synthesis and antimicrobial activity against bee pathogens (Sabaté et al. 2009). The aim of the current work was to study the effect of surfactin on the food-borne pathogen *L. monocytogenes*. Thus, bacteriocin-resistant and sensitive *L. monocytogenes* strains were analyzed (Audisio et al. 2005; Ibarguren et al. 2010). Also, the surfactin stability at different pH values, after heating and treating with proteolytic enzymes, was determined to evaluate its

* Corresponding author. Tel.: +54 387 4251006; fax: +54 387 4251006.
E-mail address: audisio@unsa.edu.ar (M.C. Audisio).

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Table 1
Listeria monocytogenes strains used as indicators.

Strain	Origin
Listeria monocytogenes 99/287[a]	INEI-ANLIS, Malbrán
Listeria monocytogenes 99/287 RB6[b]	Audisio et al. (2005)
Listeria monocytogenes 99/287 RC1	Audisio et al. (2005)
*Listeria monocytogenes*99/287 RMori1	Ibarguren et al. (2010)
*Listeria monocytogenes*99/287 RJuli4b	Audisio et al. (2005)
*Listeria monocytogenes*99/287 RM2d	Ibarguren et al. (2010)
*Listeria monocytogenes*99/287 RM1b	Ibarguren et al. (2010)

[a] GenBank access code JN086993.
[b] GenBank access code JN086995.

possible incorporation at a certain stage in the food production chain.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Bacillus subtilis C4, M1 and G2III, selected and characterized phylogenetically in previous studies (Sabaté et al. 2009), were activated in BHI broth (Brain-Heart-Infusion, Britania, Argentina) at 37 °C for 24 h.

Seven *L. monocytogenes* strains, six of which were resistant to different bacteriocins, were used as indicator strains of antimicrobial activity (Audisio et al. 2005; Ibarguren et al. 2010) (Table 1). These strains were also activated in BHI broth at 37 °C and incubated for 24 h.

2.2. Phylogenetic characterization of the Bacillus subtilis strains at sub-species level

DNA extraction was carried out with an active culture after incubation in 5 mL of BHI broth at 37 °C for 24 h according to Miller (1972). *B. subtilis gyrA* gene fragments were amplified with PCR using *gyrA*-f (5-CAGTCAGGAAATGCGTACGTCCTT-3) and *gyrA*-r (5-CAAGGTAATGCTCCAGGCATTGCT-3) primers (Kunst et al. 1997). Polymerase chain reaction (PCR) was carried out as follows: DNA was amplified in 25 μL reaction mixture containing: 0.2 μL Taq polymerase, 2.5 μL STR 10× buffer, 0.1 μL of each primer, 17.5 μL of water for PCR and 5 μL of the DNA template. Amplification consisted of an initial denaturation step at 94 °C for 2 min; 40 cycles of 94 °C for 30 s, 51 °C for 45 s and 72 °C for 60 s; and a final extension at 72 °C for 7 min (de Clerck et al. 2004).

The PCR products were separated with 0.8% (w/v) agarose gel electrophoresis at 70 V for 60 min. The gels were stained with ethidium bromide and visualized with UV, and the PCR product was sequenced at Macrogen (Seoul, Korea). On-line search for similarity was carried out at GenBank using the BLAST programme (http://www.ncbi.nlm.nih.gov).

2.3. Surfactin fraction

Surfactin was synthesized by *B. subtilis* C4, M1 and G2III and obtained by acidic precipitation and subsequent recovery with methanol according to Sabaté et al. (2009). The precipitate containing the metabolite was dissolved in sterile distilled water (pH 9) at a final concentration of 1 mg/mL. This we called the surfactin fraction.

2.4. Anti-Listeria activity

2.4.1. Qualitative analysis

The anti-*Listeria* effect was analyzed using cell-free supernatant (CFS) and surfactin obtained from the different *Bacillus* strains using the plate diffusion technique (Tagg and McGiven 1971) and modified by Audisio (2005). CFS was recovered by centrifugation (10,000 *g* for 10 min at 4 °C) and afterwards filter-sterilized (0.45 μm cellulose acetate membrane). Therefore, aliquots of CFS or surfactin of different concentrations (23 μL) were poured into wells punched in the lawn of the indicator strain and the plates were incubated at 37 °C for 24 h. The lawn was obtained by sowing 100 μL of a 24-h *L. monocytogenes* culture in 10 mL of BHI agar (1.5%, w/v). After incubation, plates were examined for the presence of inhibition halos.

2.4.2. Quantitative analysis

The effect of the CFS and surfactin produced by *B. subtilis* subsp. *subtilis* C4 on *L. monocytogenes* RB6 was quantitatively assayed with the direct contact technique (Audisio et al. 2005). Therefore, a 24 h culture of the pathogen grown in BHI was centrifuged and the pellet was recovered and subsequently resuspended in 3 mL of CFS or surfactin (1 mg/mL). The final concentration of the pathogen was the equivalent of 0.5 on the MacFarland scale. Resuspended pathogen in peptone water was used as control.

2.5. Surfactin stability

2.5.1. Temperature effect

Heating resistance of the surfactin fraction was determined after boiling for 10 min and autoclaving at 121 °C for 15 min. Then microbial activity was assayed using the plate diffusion technique with *L. monocytogenes* 99/287 RB6 as an indicator strain. An aliquot without thermal treatment was used as control.

2.5.2. pH effect

The pH of aliquots of the surfactin fraction was adjusted to 2, 4, 6, 8 and 10 by adding 1N NaOH or HCL as appropriate. Samples were incubated at 25 °C for 1 h and the pH was measured with a pH meter (Altronix TPX II, Argentina). Later, antimicrobial activity was examined with the plate diffusion technique against *L. monocytogenes* 99/287RB6. Sterile culture medium aliquots were adjusted to the same pH values assayed and used as controls.

2.5.3. Effect of proteolytic enzymes

One aliquot of the surfactin fraction was added to a final enzyme concentration of 1 mg/mL of pepsin, trypsin, α-chemotrypsin, proteinase K and pronase E (purchased from Sigma) at 37 °C for 1 h. The reaction was stopped by inactivation of the enzymes by heating to 96 °C for 10 min. All enzyme solutions were prepared in 0.05 M phosphate buffer, pH 7.0. Then, antimicrobial activity was measured with the plate diffusion technique against *L. monocytogenes* 99/287RB6, as indicator strain. Fractions without enzyme treatment were used as control.

3. Results

3.1. Characterization of Bacillus subtilis at sub-species level using gyrA gene sequencing

The *gyrA* gene codes for the A subunit of DNA gyrase in *Bacillus* strains. When *gyrA* gene sequencing was carried out it was found that *B. subtilis* C4, G2III and M1 showed 97, 97 and 99% homology, respectively, with the DNA gyrase sequence of *B. subtilis* subsp. *subtilis* 168 from the GenBank database using the BLAST programme. The *gyrA* nucleotide sequences of the strains were deposited into the GenBank with accession numbers HQ828992, HQ828991 and HQ828990, respectively. The genetic similarity of the strains can be observed in the phylogenetic tree given in Fig. 1. The phylogenetic

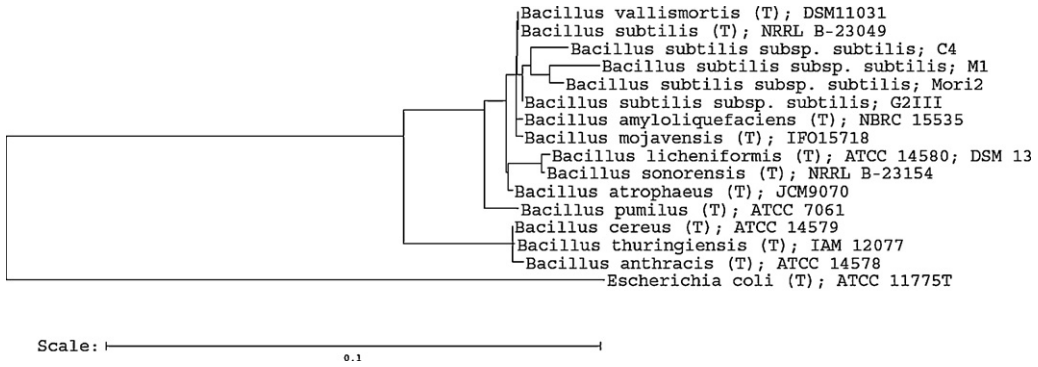

Fig. 1. Phylogenetic tree, based in the *gyrA* gen sequences of the three *B. subtilis* subsp. *subtilis*: C4, M1 and G2III strains, was constructed using the Tree Builder program of the Ribosomal Data Base Project II (http://rdp.cme.msu.edu).

distance of these strains with human pathogens of the *Bacillus* genus, like *Bacillus cereus* and *Bacillus anthracis*, should be stressed.

3.2. Antimicrobial properties

Using the plate diffusion technique it could be observed that the CFS and surfactin fractions obtained from the three *B. subtilis* subsp. *subtilis* strains showed anti-*Listeria* activity (Fig. 2). The listericide effect was strain dependant. The surfactin synthesised by *B. subtilis* subsp. *subtilis* C4 inhibited the pathogen with a concentration of 0.125 mg/mL while 0.25 mg/mL of the surfactin produced by *B. subtilis* subsp. *subtilis* G2III was necessary to inhibit *L. monocytogenes*. The lowest antagonistic effect was observed for *B. subtilis* subsp. *subtilis* M1, which only inhibited the pathogen with 1 mg/mL of surfactin.

B. subtilis subsp. *subtilis* C4 was selected for quantitative analysis due to its anti-*Listeria* activity. Contact of *L. monocytogenes* 99/287 RB6 with CFS of the *Bacillus* strain diminished pathogen viability 2 log orders and surfactin decreased viability approximately 5 log orders after 24 h of contact (Fig. 3).

3.3. Stability of the antibacterial activity of surfactin

When the effect of the proteolytic enzymes on the antibacterial activity of surfactin was assayed it was found that under the given assay conditions none of the enzymes affected stability, because the metabolite did not show loss of its inhibitory activity against *L. monocytogenes* 99/287RB6 (Table 2).

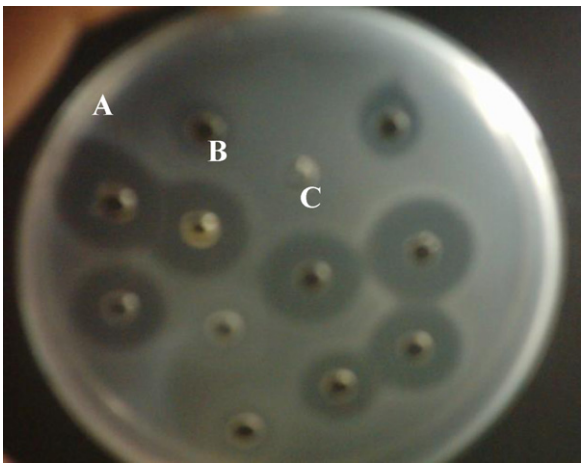

Fig. 2. Anti-listeria activity from surfactin synthesized by *B. subtilis* subsp. *subtilis* (A) C4, (B) M1 and (C) G2III.

Surfactin synthesized by the different *Bacillus subtilis* strains was also stable within the pH range assayed (2–10), as the metabolite maintained its inhibitory effect against the pathogen. Extreme temperatures, boiling for 10 min and autoclaving at 121 °C for 15 min, had no affect on this biosurfactant (Table 3).

4. Discussion

Bacteria belonging to *Bacillus subtilis* can be divided into two sub-species: *B. subtilis* subsp. *subtilis* and *B. subtilis* subsp. *spizizenii* (Nakamura et al. 1999). Genes that codify proteins present a much higher genetic variability among species than other genes, therefore they are highly suitable for classification and identification of closely related species (Mollet et al. 1997; Kim et al. 1999; Yamamoto et al. 1999). Sequencing of the *gyrA* gene, which codifies the A sub-unit of DNA gyrase in *Bacillus*, is useful to discriminate between species most closely related to *B. subtilis* and also between sub-species (Chun and Bae 2000; Palmisano et al. 2001). *B. subtilis* C4 and M1, isolated from honey, and G2III, isolated from bee gut (Sabaté et al. 2009), were characterized as *B. subtilis* subsp. *subtilis* after *gyrA* gene sequencing at sub-species level as they presented 97, 99 and 97% homology, respectively, with the DNA gyrase sequence of *B. subtilis* subsp. *subtilis* 168, strain previously phylogenetically characterized and published by Kunst et al. (1997) (accession number AL009126). There are only a few scientific articles that have characterized and identified surfactin-producing *B. subtilis* subsp. *subtilis* strains. Geetha et al. (2010) isolated a soil bacterium identified as *B. subtilis* subsp. *subtilis*, which is able to synthesise different surfactin isoforms. However, to date, no antecedents exist regarding surfactin synthesis by *B. subtilis* subsp. *subtilis* strains isolated from the honey and/or bee environment.

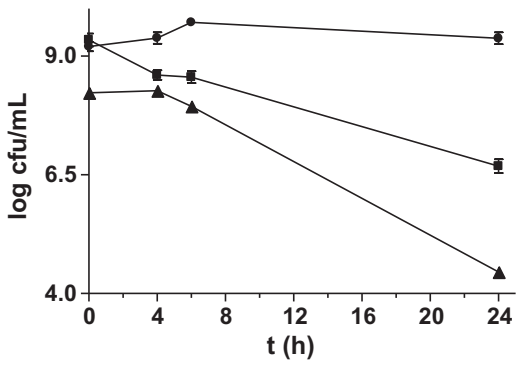

Fig. 3. Effect of cell-free supernatant (CFS) (■) and surfactin (▲) from *B. subtilis* subsp. *subtilis* C4 on *L. monocytogenes* 99/287RB6 viability. Control of *L. monocytogenes* 99/287RB6 cells (●).

Table 2
Effect of proteolytic enzymes on anti-*Listeria* activity from surfactin.

Enzymes (1 mg/mL)	Surfactin		
	B. subtilis subsp. *subtilis* C4	*B. subtilis* subsp. *subtilis* G2III	*B. subtilis* subsp. *subtilis* M1
Surfactin without enzyme treatment	12[a]	10	10
Pepsin	11	10	9
Trypsin	11	10	8
α-Chemotrypsin	10	10	9
Pronase E	10	8	10
Proteinase K	10	9	9

[a] Inhibition halo diameter (mm) against *L. monocytogenes* 99/287RB6.

Table 3
The stability of surfactin in extreme conditions.

Strain	Temperature effect		pH effect
	Boiling (96 °C, 10 min)	Autoclave (121 °C, 15 min)	
B. subtilis subspp. *subtilis* C4	+	+	+ (2–10)[a]
B. subtilis subspp. *subtilis* M1	+	+	+ (2–10)
B. subtilis subspp. *subtilis* G2III	+	+	+ (2–10)

(+) Positive activity on *L. monocytogenes* 99/287RB6 by the well-diffusion assay.
[a] pH range where the inhibitory action is constant.

L. monocytogenes is an emerging pathogen with a strong impact on pregnant women, children and elderly people and its virulence has increased three hundred-fold in AIDS patients (Jurado et al. 1993; Hitchins and Whiting 2001). The inhibition or control of this microorganism by bacteria belonging to *Bacillus* spp. has been reported (Bizani et al. 2005; Lisboa et al. 2006; Sharma et al. 2006; Cherif et al. 2008; Liao 2009; Spathelf and Rautenbach 2009; Tabbene et al. 2010). Interestingly, none of these bacilli with anti-*Listeria* activity were isolated from honey or bees and the compounds with the antagonistic effects were mainly different bacteriocins produced by *B. cereus*, *Bacillus thuringiensis*, *Bacillus amyloliquefaciens* and *Bacillus lentus* (Bizani et al. 2005; Cherif et al. 2008; Lisboa et al. 2006; Sharma et al. 2006, respectively), or tyrocidine by *Bacillus aneurinolyticus* (Spathelf and Rautenbach 2009). As can be observed, all these compounds were different from surfactin. Only Tabbene et al. (2010) worked with a *B. subtilis* strain, but the chemical nature of the anti-*Listeria* compound was not completely determined and did not suggest that it could be surfactin. All the authors previously mentioned determined the antagonistic effect using only one strain of this pathogen, except for Spathelf and Rautenbach (2009), who used two *L. monocytogenes* strains, one of which was resistant to bacteriocins. However, *B. aneurinolyticus* did not produce surfactin as the compound with the antilisteria effect.

As can be seen, few studies exist on the antagonistic activity of surfactin synthesized by *B. subtilis* against another human pathogenic bacteria. Hwang et al. (2007) found that surfactin C reduced the number of *Escherichia coli* cells in mice infected with this pathogen. Huang et al. (2007) reported on the antagonistic effect of surfactin on *B. cereus* spores and recommended the use of this metabolite as an additive in food products to prevent the pathogen's growth. Nitschke et al. (2009) and Zeraik and Nitschke (2010) studied the effect of surfactin synthesized by a *B. subtilis* strain isolated from soil. They analyzed the effect of the compound on adhesion properties of *L. monocytogenes* to stainless steel and polypropylene surfaces and observed that surfactin diminished adhesion of the pathogen. de Araujo et al. (2011) also found that surfactin reduced the adhesion of the pathogen to polystyrene surfaces, but they did not report any significant effect of this metabolite on *L. monocytogenes* viability.

In this work, it has been determined that surfactin synthesized by *B. subtilis* subsp. *subtilis* C4, M1 and G2III exerted an important anti-*Listeria* effect, not only on "common" *L. monocytogenes* strains but also on strains that have become irreversibly resistant to enterocins (Ibarguren et al. 2010). These enterocins are produced by *Enterococcoccus avium* PA1 (Audisio et al. 2005). The importance of this antibacterial activity should be emphasized because only one research paper has addressed this topic before (de Araujo et al. 2011) and the authors determined that surfactin showed only a limited inhibitory activity against *L. monocytogenes*.

Surfactins produced by microorganisms have several advantages over those chemically synthesised: they present less toxicity and higher biodegradability and they are generally able to maintain antagonistic activity at extreme temperature and pH (Kosaric 1992; Singh and Cameotra 2004; Gautam and Tyagi 2006; Nitschke and Costa 2007; Abdel-Mawgoud et al. 2008; Ghojavand et al. 2008). Therefore, biosurfactants have been proposed as novel additives in the food production to combat or prevent pathogen growth (Nitschke and Costa 2007). The current study shows that surfactin produced by the three *Bacillus* strains, which were previously chemically characterized (Sabaté et al. 2009), not only maintains antibacterial activity at different pH values, and after heat treatment, but it is also active after treatment with proteolytic enzymes.

This is the first study that has determined anti-*Listeria* activity of surfactin against different *L. monocytogenes* strains, mainly against those bacteriocin-resistant. The results obtained suggest that surfactin, a microbial compound, could be considered another natural tool in the development of new strategies to prevent or control *L. monocytogenes* in the food industry.

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References

Abdel-Mawgoud AM, Aboulwafa MM, Hassouna NA. Characterization of surfactin produced by *Bacillus subtilis* isolate BS5. Appl Biochem Biotechnol 2008;150:289–303.

Al-Ajlani MM, Sheikh MA, Ahmad Z, Hasnain S. *Bacillus subtilis* strains produce a broad spectrum of bioactive peptides with great potential for biotechnological and biopharmaceutical applications. Microb Cell Fact 2007;6:17.

Audisio MC, Terzolo HR, Apella MC. Bacteriocin from honeybee beebread *Enterococcus avium* active against *Listeria monocytogenes*. Appl Environ Microbiol 2005;71:3373–5.

Bai Y, D'Aoust F, Smith DL, Driscoll BT. Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. Can J Microbiol 2002;48:230–8.

Bizani D, Motta AS, Morrissy JA, Terra RM, Souto AA, Brandelli A. Antibacterial activity of cerein 8A, a bacteriocin-like peptide produced by *Bacillus cereus*. Int Microbiol 2005;8:125–31.

Cherif A, Rezgui W, Raddadi N, Daffonchio D, Boudabous A. Characterization and partial purification of entomocin 110, a newly identified bacteriocin from *Bacillus thuringiensis* subsp. *entomocidus* HD110. Microbiol Res 2008;163:684–92.

Chun J, Bae KS. Phylogenetic analysis of *Bacillus subtilis* and related taxa based on partial gyrA gene sequences. Antonie Leeuwenhoek 2000;78:123–7.

Cutting SM. Bacillus probiotics. Food Microbiol 2011;28:214–20.

Czaczyk K, Stachowiak B, Trojanowska K, Gulewicz K. Antifungal activity of *Bacillus* sp. isolated from compost. Folia Microbiol (Praha) 2000;45:552–4.

de Araujo VL, Abreu F, Lins U, Santa Anna LMM, Nitschke M, Guimarães Freire DM. Rhamnolipid and surfactin inhibit *Listeria monocytogenes* adhesion. Food Res Int 2011;44:481–8.

de Clerck E, Vanhoutte T, Hebb T, Geerinck J, Devos J, de Vos P. Isolation, characterization, and identification of bacterial contaminants in semifinal gelatin extracts. Appl Environ Microbiol 2004;70:3664–72.

Etchegaray A, de Castro Bueno C, de Melo IS, Tsai SM, Fiore MF, Silva-Stenico ME, et al. Effect of a highly concentrated lipopeptide extract of *Bacillus subtilis* on fungal and bacterial cells. Arch Microbiol 2008;190:611–22.

Fiechter A. Biosurfactants: moving towards industrial application. Trends Biotechnol 1992;10:208–17.

Gandhi M, Chikindas ML. Listeria: a foodborne pathogen that knows how to survive. Int J Food Microbiol 2007;113:1–15.

Gautam KK, Tyagi VK. Microbial surfactants: a review. J Oleo Sci 2006;55:155–66.

Geetha I, Manonmani AM, Paily KP. Identification and characterization of a mosquito pupicidal metabolite of a *Bacillus subtilis* subsp. *subtilis* strain. Appl Microbiol Biotechnol 2010;6:1737–44.

Ghojavand H, Vahabzadeh F, Roayaei E, Shahraki AK. Production and properties of a biosurfactant obtained from a member of the *Bacillus subtilis* group (PTCC 1696). J Colloid Interface 2008;324:172–6.

Hitchins AD, Whiting RC. Food-borne *Listeria monocytogenes* risk assessment. Food Addit Contam 2001;18:1108–17.

Holzapfel WH, Geisen R, Schillinger U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int J Food Microbiol 1995;24:343–62.

Huang XQ, Lu ZX, Bie XM, Lu FX, Zhao HZ, Yang SJ. Optimization of inactivation of endospores of *Bacillus cereus* by antimicrobial lipopeptides from *Bacillus subtilis* fmbj strains using a response surface method. Appl Microbiol Biot 2007;74:454–61.

Hwang YH, Park BK, Lim JH, Kim MS, Park SC, Hwang MH, et al. Lipopolysaccharide-binding and neutralizing activities of surfactin C in experimental models of septic shock. Eur J Pharmacol 2007;556:166–71.

Ibarguren C, Raya RR, Apella MC, Audisio MC. *Enterococcus faecium* isolated from honey synthesized bacteriocin-like substances active against different *Listeria monocytogenes* strains. J Microbiol 2010;48:44–52.

Joo GJ, Kim YM, Lee IJ, Song KS, Rhee IK. Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides* and *Bacillus pumilus*. Biotechnol Lett 2004;26:487–91.

Jurado RL, Farley MM, Pereira E, Harvey RC, Schuchat A, Wenger JD, et al. Increased risk of meningitis and bacteremia due to *Listeria monocytogenes* in patients with human inmunodeficiency virus infection. Clinic Infect Dis 1993;17:224–7.

Kim BJ, Lee SH, Lyu MA, Kim SJ, Bai GH, Chae GT, et al. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (rpoB). J Clin Microbiol 1999;37:1714–20.

Kosaric N. Biosurfactants in industry. J Am Oil Chem Soc 1992;64:1731–7.

Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azevedo V, et al. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. Nature 1997;390:249–56.

Liao CH. Control of foodborne pathogens and soft-rot bacteria on bell pepper by three strains of bacterial antagonists. J Food Prot 2009;72:85–92.

Lisboa MP, Bonatto D, Bizani D, Henriques JA, Brandelli A. Characterization of a bacteriocin-like substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic forest. Int Microbiol 2006;9:111–8.

Manachini PL, Fortina MG, Parini C. Characterization of a crude proteolytic preparation of potential application. Ann Microbiol Enzymol 1987a;37:145–50.

Manachini PL, Parini C, Fortina MG, Benazzi L. BliI, a restriction endonuclease from *Bacillus licheniformis*. FEBS Lett 1987b;214:305–7.

Manachini PL, Fortina MG, Parini C. Enzymic modification of vegetable protein by a crude preparation from a strain of *Bacillus licheniformis*. J Sci Food Agric 1988;45:263–6.

Mannanov RN. The use of natural bio-agents for the control of cotton phytopathogens. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet 2001;66:183–6.

McLauchlin J, Mitchell RT, Smerdon WJ, Jewell K. *Listeria monocytogenes* and listeriosis: a review of hazard characterization for use in microbiological risk assessment of foods. Int J Food Microbiol 2004;92:15–33.

Miller JH. Experiments in molecular genetics. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory; 1972. p. 466.

Ming X, Daeschel MA. Nisin resistance of foodborne bacteria and the specific resistance responses of *Listeria monocytogenes* Scott A. J Food Prot 1993;56:944–8.

Mollet C, Drancourt M, Raoult D. rpoB sequence analysis as a novel basis for bacterial identification. Mol Microbiol 1997;26:1005–11.

Nakamura LK, Roberts MS, Cohan FM. Relationship of *Bacillus subtilis* clades associated with strains 168 and W23: a proposal for *Bacillus subtilis* subsp. *subtilis* subsp. *nov*. and *Bacillus subtilis* subsp. *spizizeni* subsp. *nov*. Int J Syst Evol Microbiol 1999;49:1211–5.

Nitschke M, Costa SG. Biosurfactants in food industry. Trends Food Sci Tech 2007;18:252–9.

Nitschke M, Araújo LV, Costa SG, Pires RC, Zeraik AE, Fernandes AC, et al. Surfactin reduces the adhesion of food-borne pathogenic bacteria to solid surfaces. Lett Appl Microbiol 2009;49:241–7.

Ongena M, Jacques P. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol 2008;16:115–25.

Palmisano MM, Nakamura LK, Duncan KE, Istock CA, Cohan FM. *Bacillus sonorensis* sp. *nov*., a close relative of *Bacillus licheniformis*, isolated from soil in the Sonoran Desert, Arizona. Int J Syst Evol Microbiol 2001;51:1671–9.

Raaijmakers JM, de Bruijn I, Nybroe O, Ongena M. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS Microbiol 2010;34:1037–62.

Sabaté DC, Carrillo L, Audisio MC. Inhibition of *Paenibacillus larvae* and *Ascosphaera apis* by *Bacillus subtilis* isolated from honeybee gut and honey samples. Res Microbiol 2009;160:163–9.

Sharma N, Kapoor G, Neopaney B. Characterization of a new bacteriocin produced from a novel isolated strain of *Bacillus lentus* NG121. Antonie Leeuwenhoek 2006;89:337–43.

Singh P, Cameotra SS. Potential applications of microbial surfactants in biomedical sciences. Trends Biotechnol 2004;22:142–6.

Spathelf BM, Rautenbach M. Anti-listerial activity and structure-activity relationships of the six major tyrocidines, cyclic decapeptides from *Bacillus aneurinolyticus*. Bioorgan Med Chem 2009;17:5541–8.

Tabbene O, Karkouch I, Elkahoui S, Cosette P, Mangoni ML, Jouenne T, et al. A new antibacterial and antioxidant S07-2 compound produced by *Bacillus subtilis*áB38. FEMS Microbiol Lett 2010;303:176–82.

Tagg JR, McGiven AR. Assay system for bacteriocins. Appl Microbiol 1971;21:943.

Vadyvaloo V, Snoep JL, Hastings JW, Rautenbach M. Physiological implications of class IIa bacteriocin resistance *Listeria monocytogenes* strains. Microbiology 2004;150:335–40.

Velmurugan N, Choi MS, Han SS, Lee YS. Evaluation of antagonistic activities of *Bacillus subtilis* and *Bacillus licheniformis* against wood-staining fungi: in vitro and in vivo experiments. J Microbiol 2009;47:385–92.

Yamamoto S, Bouvet PJ, Harayama S. Phylogenetic structures of the genus *Acinetobacter* based on gyrB sequences: comparison with the grouping by DNA-DNA hybridization. Int J Syst Bacteriol 1999;49:87–95.

Zeraik AE, Nitschke M. Biosurfactants as agents to reduce adhesion of pathogenic bacteria to polystyrene surfaces: effect of temperature and hydrophobicity. Curr Microbiol 2010;61:554–9.

Zukowski MM. Production of commercially valuable products. In: Doi RH, McGoughlin M, editors. Biology of bacilli, applications to industry. Boston: Butterworth-Heinemann; 1992. p. 311–37.