Probiotics & Antimicro. Prot. (2010) 2:120–125
DOI 10.1007/s12602-010-9040-9

Antilisterial Activity on Poultry Meat of Amylolysin, a Bacteriocin from *Bacillus amyloliquefaciens* GA1

Badre Halimi · Carine Dortu · Anthony Arguelles-Arias · Philippe Thonart · Bernard Joris · Patrick Fickers

Published online: 19 March 2010

Abstract This paper describes the production, the purification and the antilisterial activity of amylolysin, a novel bacteriocin from *B. amyloliquefaciens* GA1. The strain genome was first analysed using PCR techniques for the presence of gene clusters that direct the synthesis of characterised bacteriocins from *B. amyloliquefaciens* and the closely related *B. subtilis*. Our results suggest that amylolysin corresponds to a novel bacteriocin. The effect of amylolysin on the growth of different isolates of *Listeria monocytogenes* was evaluated in poultry meat during 21 days of storage at 4 °C. A potent antilisterial effect was observed for all the indicator strains tested, demonstrating that amylolysin is a novel bacteriocin that could be used as a food preservative.

Keywords Bacteriocin · Bacillus amyloliquefaciens · Listeria monocytogenes

Carine Dortu and Badre Halimi have contributed equally to this work.

B. Halimi · A. Arguelles-Arias · B. Joris · P. Fickers
Center for Protein Engineering (CIP), University of Liège, Bldg. B6a, Allée de la chimie, 4000 Liège, Belgium

B. Halimi · C. Dortu · P. Thonart
Walloon Center for Industrial Biology, University of Liège, Bldg. 40, Bd du Rectorat, 4000 Liège, Belgium

P. Fickers (✉)
BioTechnology and BioProcess, 3Bio Department, Université Libre de Bruxelles, Av F.-D. Roosevelt, 50, CP165/61, 1050 Brussels, Belgium
e-mail: pfickers@ulb.ac.be

Introduction

Listeria monocytogenes is the causative agent of food-related listeriosis especially in newborn, elderly and immunocompromised patients [25]. This bacterium has the ability to grow in vacuum-packaged food at chiller temperatures and is relatively tolerant to salt and low pH, rendering its control in food difficult. *L. monocytogenes* is the main food-borne pathogen causing the highest mortality rate, far ahead of *Campylobacter*, *Salmonella* or *Escherichia coli* [21]. Consequently, its control in food has become a major concern in the food industry. To minimise the risk of such a bacterial development in food, synthetic preservatives are used in addition to refrigeration. However, the safety of these compounds has been more and more questioned in recent years, increasing consumer demand for natural products as alternative food preservatives.

Bacteriocins are proteinaceous antimicrobial compounds synthetised by many bacteria, with some active against a wide range of bacteria [22]. Nisin from *Lactococcus lactis* is to date the most studied antimicrobial peptide (for review see [19]). This bacteriocin has been attributed GRAS status and is used commercially as a food preservative in more than 40 countries [8]. However, its inactivation in certain types of food and the emergence of nisin-resistant strains point out the need for the characterisation of new bacteriocins or bacteriocin-like compounds to overcome these problems [5, 15, 18].

Most of the bacteriocins studied are from lactic acid bacteria because they are commonly associated in food, especially in meat and dairy products [5, 11]. However, *Bacillus* is an alternative genus to investigate for antimicrobial peptides, because it includes many industrial species and has a history of safe use in the food industry [23].

Several bacteriocins or related substances produced by *Bacillus* have been reported. These include subtilin from *B. subtilis* [17], megacin A-216 from *B. megaterium* [16], cerein 7 from *B. cereus* [4] and subtilosin A, produced by some strains of both *B. subtilis* and *B. amyloliquefaciens* [3, 4, 24]. Some of these substances have been successfully tested as food preservatives in dairy products [4].

Recently, we have isolated a proteinaceous compound from *B. amyloliquefaciens* GA1 which presents an antimicrobial activity against several Gram positive bacteria, including *L. monocytogenes* (Fickers et al., unpublished). The objective of the present study was to produce and evaluate the ability of this bacteriocin, named amylolysin, to inhibit the growth of *L. monocytogenes* in poultry meat.

Materials and Methods

Strains, Media and General Genetic Techniques

Bacillus amyloliquefaciens GA1 was isolated from strawberry and identified in our laboratory by sequencing *recA* and *recN* genes [2]. The *Listeria* isolates used as indicator strains are listed in Table 1. Luria–Bertani (LB), brain–heart (BH) and PALCAM media were of commercial origin (Becton–Dickinson, Le Pont de Chaix, France), whereas CAT medium composition was as described elsewhere [6]. Genomic DNA was purified using the Wizard genomic purification kit (Promega). PCR amplifications were performed with Fideli Taq polymerase (USB Corporation, Cleveland, OH, USA) and amplified fragments were purified with the Qiagen purification kit (Hilden, Germany). Primers used for PCR amplification are listed in Table 1. DNA sequencing was performed at GIGA Genomics Facilities (University of Liège, Liège, Belgium).

Activity Spectrum of Cell-Free Supernatant

Antimicrobial activity was estimated by agar diffusion assay [10]. *Listeria* strains were grown in BH liquid medium for 24 h at 37 °C. Three hundred microlitres of the resulting cell suspension was used to inoculate 120 ml of brain–heart (BH) medium containing 0.85 g of agar. Aliquots (2 × 10 µl) of *B. amyloliquefaciens* GA1 culture supernatant were applied on sterile paper discs (5 mm diameter) and deposited after drying onto BH agar plates inoculated with the Listeria indicator strain. The antilisterial activity was estimated by measuring the diameter (in mm) of the growth inhibition zone obtained after 16 h of incubation at 37 °C.

PCR Amplification of the Subtilosin, Mersacidin and Subtilin Structural Genes

Subtilin, subtilosin A, sublancin 168, mersacidin, ericin A and ericin S structural genes were PCR-amplified with primer pairs spaSfo/spoSrev, sboAfo/sboArev, sunAfo/sunArev, mrsAfo/mrsArev and eriAfo/eriSrev, respectively (Table 2), using genomic DNA from *B. amyloliquefaciens* GA1 strain as a template.

Culture of *B. amyloliquefaciens* GA1 and Amylolysin Production

Cell growth and amylolysin production were monitored in LB and CAT media. Growth was observed at 37 °C in 400 ml of medium inoculated at an initial cell concentration of 10^3 CFU ml^{-1}. At specific time intervals, samples were collected. Cell growth was monitored either by determining the optical density at 600 nm or by cell dry weight determination. Amylolysin concentration in the culture supernatant was determined by reverse-phase high performance liquid chromatography (RP-HPLC, see below) after extraction using Chromafix C_{18} EC cartridges (Macherey–Nagel, Duren, Germany). Growths were performed in triplicate. Large-scale production of amylolysin was performed in a Bioflo 4500 bioreactor (New Brunswick, Edison, NJ, USA) with a working volume of 15 l. Seeding was performed at an initial cell concentration of 10^3 CFU ml^{-1}. Experiments were conducted for 6 h at 37 °C at a stirring speed of 250 rpm and an aeration flow of 0.5 VVM (volume of air per volume of medium per minute). Cell-free supernatant was obtained by cross flow filtration using a Hollow fibre cartridge (0.45 µm, 8400 cm^2,

Table 1 Activity spectrum of cell-free culture supernatant of *B. amyloliquefaciens* GA1

Strain	Zone of inhibition (diameter in mm)	Origin, reference
L. monocytogenes LMG23905	20	Poultry, [9]
L. monocytogenes LMG21263	24	Poultry, [9]
L. monocytogenes LM2234	20	Fish, [9]
L. innocua ATCC33090[a]	14	Cow brain
L. innocua RFB159	14	Laboratory stock
L. ivanovii RFB160	16	Laboratory stock

[a] American Type Culture Collection

Table 2 Synthetic primers used for PCR amplification

Name	Sequence (5′-3′)
spaSfo	CAAAGTTCGATGAATTCGATTTGGATGT
spaSrev	GCAGTTACAAGTTAGTGTTTGAAGGAA
sboAfo	CGCGCAAGTAGTCGATTTCTAACA
sboArev	CGCGCAAGTAGTCGATTTCTAACA
mrsAfo	GGGTATATGCGGTATAAACTTATG
mrsArev	GTTTCCCCAATGATTTACCCTC
sunAfo	GAGGAACTCGAAAACCAAAAAGG
sunArev	GGAATTGACTCTTTGTCAATTTC
eriAfo	GTGACTAATATGTCAAAGTTCGATG
eriSrev	GAGATATGGCAGTTACAAGTAATTG

GE Healthcare, Uppsala, Sweden) according to the manufacturer's recommendations.

Purification and Quantification of Amylolysin

Amylolysin purification was performed by hydrophobic chromatography using Amberlite XAD-16 resin (Sigma–Aldrich, St. Louis, USA). The resin was dispersed in the growth supernatant (40 g per litre) and stirred at 4 °C for 4 h before being washed successively with 5 volumes of water and 5 volumes of a water/methanol mixture (1:1 v/v). The bacteriocin was eluted with two volumes of methanol and concentrated by rotary evaporation at 50 °C. Amylolysin concentration was determined by RP-HPLC using a Nucleodur C18 column (250 × 4.6 mm, 5 µm packing, Macherey–Nagel, Duren, Germany) and an acetonitrile/H_2O/trifluoroacetic acid mixture (41.5/59/0.5, v/v/v) as mobile phase. Samples (100 µl) were eluted at a flow rate of 1 ml min^{-1}. Purified nisin (Molekula, Dorset, UK) was used as a standard for quantification. Amylolysin was identified based on the second derivatives of its UV–visible spectra (Waters PDA 996 diode array; Millenium Software, Milford, MA, USA).

Protease Sensitivity

For protease sensitivity, purified amylolysin (100 ng) was incubated with pronase (Fluka, St Louis, MO) at a final concentration of 1 mg ml^{-1} at 37 °C for 2 h in 0.2 M KH_2PO_4/K_2HPO_4 buffer at pH 7. Residual bacteriocin activity was then determined by the agar well diffusion assay using *L. monocytogenes* LM21263 as indicator strain. Pronase untreated samples were used as a control.

Antilisterial Activity on Chicken Meat

Chicken breast cuts were bought in a local butcher shop (Liège, Belgium). They were carved aseptically into 100 g portions and surface-inoculated with 2×10^3 CFU g^{-1} of *L. monocytogenes*. Meat pieces were sprayed with different amounts of amylolysin (5 or 10 µg g^{-1}, see Fig. 1) or nisin (10 µg g^{-1}) and stored at 4 °C in sealed bags. Samples (10 g) were taken aseptically after 0, 3, 7, 14 and 21 days of storage. They were homogenised in 10 ml of saline-peptone water (5 gl^{-1} NaCl, 1 ml l^{-1} Tween 80, 1 gl^{-1} casein peptone) for 180 s with a stomacher (Lab Blender 400, Seward, London, UK). Cell concentration of *L. monocytogenes* was determined after decimal dilution of the suspension in saline-peptone water by plating the appropriated dilutions onto PALCAM medium for 48 h at 37 °C. Samples inoculated only with *L. monocytogenes* were used as controls. Experiments were performed in triplicate and the significance of the results analysed by Student's *t*-test at $p < 0.05$.

Stability of Amylolysin Towards Meat Protease

Ten grams of poultry breast muscle was homogenised in a blender with 10 ml of 50 mM phosphate buffer pH 7.5 for 2 min at full speed. The resulting mixture was then centrifuged for 15 min at 15,000×g, and the clarified suspension was filter sterilised using a 22-µm acrodisk filter

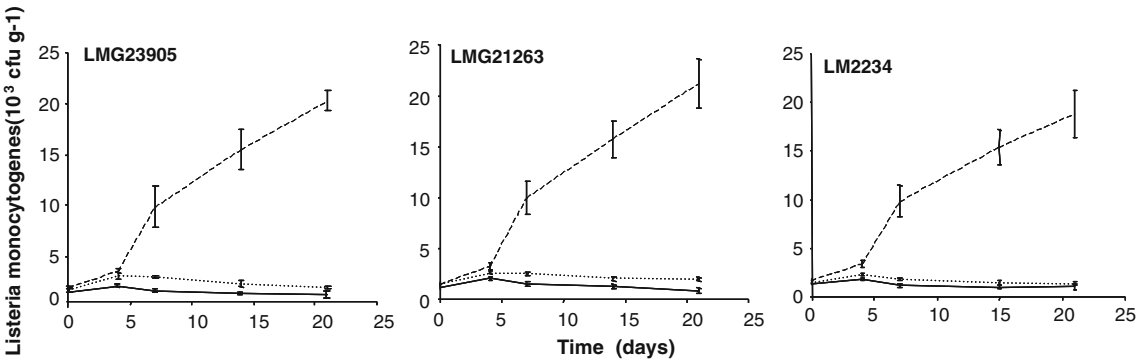

Fig. 1 Growth of *Listeria monocytogenes* LMG23905, LMG21263 and LM2234 in raw poultry meat upon storage at 4 °C in the presence of amylolysin 5 µg g^{-1} (*dotted line*), 10 µg g^{-1} (*solid line*) or in its absence (*dashed line*). Values are mean and standard deviations of three independent experiments

(Millipore, Billerica, MA, USA). Amylolysin and nisin (200 ng) were added aseptically to 1 ml of the clarified suspension. Samples containing only amylolysin or nisin in phosphate buffer were used as control. Antilisterial activity was determined by agar diffusion assay for samples incubated at 37 °C for 0, 24, 48 and 72 h using *L. monocytogenes* LM21263 as indicator strain. The remaining antimicrobial activity was defined as the ratio of the diameter of the growth inhibition zone obtained for the treated samples to that obtained for control samples. Experiments were performed in triplicate and the significance of the results analysed by Student's *t*-test at $p < 0.05$.

Results

Activity Spectrum of Cell-Free Supernatant

The susceptibility to amylolysin of six *Listeria* isolates from various origins was evaluated. As shown in Table 1, the cell-free supernatant of *B. amyloliquefaciens* GA1 was determined to have similar antimicrobial activity against the three *L. monocytogenes* isolates tested. Inhibitory activity was also observed towards *L. innocua* and *L. ivanovii* isolates, although to a lesser extent.

Genetic Analysis

Polymerase chain reactions were performed to assess the relatedness of amylolysin to the different known bacteriocins from the *Bacillus* genera. PCR amplifications were performed on strain GA1 genomic DNA using primer pairs designed according to the corresponding structural gene sequence available in GenBank. The absence of PCR amplification products indicated that *B. amyloliquefaciens* GA1 is negative for the genes encoding subtilin (*spaS*), subtilosin A (*sboA*), sublancin 168 (*sunA*), mersacidin (*mrsA*), ericin A (*eriA*) and ericin S (*eriS*) (data not shown). This suggests that the antilisterial compound produced by strain GA1 does not correspond to any of these bacteriocins.

Production and Purification of Amylolysin

Amylolysin production was first investigated by growing strain GAI in shaking flasks containing either complex (LB) or defined (CAT) medium. Cell growth and amylolysin production were determined at different time periods over 24 h. In both cases, the maximal amylolysin concentration was obtained at the end of the growth phase. It decreased thereafter during the stationary phase (data not shown). For culture performed in CAT medium, very low amylolysin productivity was obtained (0.09 mg/l/mg DW) compared to complex medium (2.51 mg/l/mg DW) (Table 3). Based on these observations, large-scale production of amylolysin in a bioreactor was performed in LB medium for 6 h at 37 °C. During the culture, a slight medium acidification from pH 7.5–6.9 could be observed and dissolved oxygen was never below 30% of saturation, indicating that the culture was never in anoxia under these conditions (data not shown). The maximal amylolysin productivity (4.83 mg/l/mg DW), obtained after 6 h of growth, represents a 1.8-fold increase compared to that obtained in flask culture. The purification of the bacteriocin from the 15 l of growth supernatant was conducted by hydrophobic chromatography. After adsorption and subsequent desorption on Amberlite XAD-16 resin, the purified bacteriocin was concentrated and quantified by RP-HPLC using nisin as a reference. From the 15 l of culture supernatant, 120 mg of purified amylolysin was recovered. The loss of 75% of the antilisterial activity after pronase treatment of the purified amylolysin demonstrated its proteinaceous nature (data not shown).

Table 3 Amylolysin concentration in cell-free culture supernatant of *B. amyloliquefaciens* GA1

Medium, culture condition	Productivity (mg/l/mg DW)	Time[a] (h)
CAT, flask	0.09	8
LB, flask	2.51	6
LB, bioreactor	4.83	6

DW dry weight

[a] Refers to the time culture when amylolysin concentration was maximal

Effect of Amylolysin on *Listeria monocytogenes* Growth in Poultry Meat

Chicken breast cuts were used to evaluate the effect of amylolysin on the growth of three different *L. monocytogenes* isolates. In control samples, the population of the indicator strains increased by at least a log of magnitude after 21 days of storage at 4 °C (Fig. 1). Indeed, the initial *L. monocytogenes* population (2×10^3 CFU g^{-1}) increased to a level of 2.0×10^4, 2.1×10^4 and 1.8×10^4 CFU g^{-1} for strains LMG23905, LMG21263 and LM2234, respectively. By contrast, addition of amylolysin led to an inhibition of growth for the three strains tested. After 21 days of storage, plate counts showed that *L. monocytogenes* populations were equal to 1.9 10^3 CFU g^{-1}. Addition of nisin under the same conditions immediately reduced the population of *L. monocytogenes* by 0.4 log (5×10^2 CFU g^{-1}) at day 0 to 1.2 log (4×10^1 CFU g^{-1}) after 14 days and up to 21 days of storage at 4 °C (data not shown).

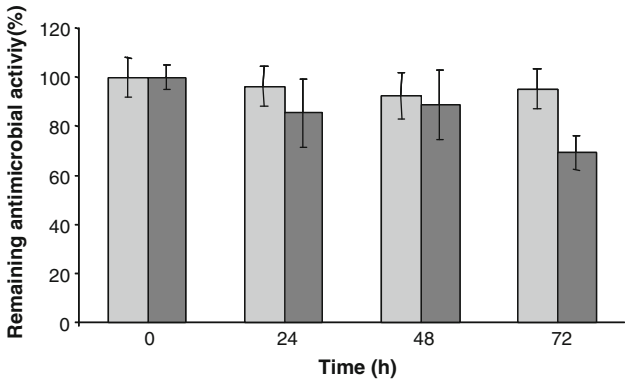

Fig. 2 Recovery of amylolysin and nisin inhibitory effect on the growth of *L. monocytogenes* LM21263 after incubation for 0, 24, 48 and 72 h with poultry meat extract. Values were normalised to those obtained in the absence of poultry meat extract. Values are mean and standard deviations of three independent experiments

Stability of Amylolysin Towards Poultry Meat Protease

Sensitivity of amylolysin to meat protease (i.e. that secreted by endogenous meat flora and that released from myocytes) was determined. Amylolysin and nisin were incubated with poultry breast muscle extract for various time periods over 72 h. The remaining ability of the two bacteriocins to inhibit the growth of *L. monocytogenes* LM21263 was then determined and normalised with values obtained for non-treated samples at the corresponding time. As shown in Fig. 2, residual inhibitory activities of amylolysin were not significantly different for any of the samples tested by contrast to nisin, which were found significantly lower after 72 h of incubation ($p < 0.05$).

Discussion

Among the few bacteriocins characterised from *Bacillus,* those from *B. amyloliquefaciens* are scarce. The only fully characterised bacteriocin from *B. amyloliquefaciens* was found to correspond to subtilosin A, a bacteriocin previously reported in *B. subtilis* [24]. However, genetic evidence suggests that this results rather from an intra-species horizontal transfer of gene (HGT) than from a mechanism selected by evolution. Other examples of HGT involving bacteriocin gene cluster were previously reported, notably for the class I bacteriocin nisin [14, 26]. The recent comparative analysis of the complete genome sequence of the reference strain *B. amyloliquefaciens* FZB42 revealed the presence of two partial gene clusters conferring resistance to the lanthionine-containing antibiotic mersacidin and subtilin [7]. The presence of these gene clusters only described to date in *B. subtilis* suggests that a similar HGT could have occurred in strain FZB42. Unfortunately, the genes involved in the synthesis of these two bacteriocins are missing, leading to a non-productive strain. However, all of these highlight *B. amyloliquefaciens* as a potential alternative organism for isolation of bacteriocin. In our laboratory, *B. amyloliquefaciens* GA1, isolated from strawberry, was selected as a producer of a heat-stable proteinous compound with potent antimicrobial activity on various Gram-positive bacteria (Fickers et al., unpublished). The antilisterial activity of this bacteriocin, named amylolysin, points to its potential utilisation as a food preservative.

The failure of structural gene detection for all the genetically described bacteriocins from *B. subtillis* strongly suggests that amylolysin is not the consequence of an HGT but rather corresponds to a novel bacteriocin not described to date. As a first characterisation, the aim of the present work was to produce and purify amylolysin in order to test its ability to inhibit the growth of the food-borne pathogen *L. monocytogenes* in meat.

It is well established that bacteriocin production undergoes a complex mechanism of regulation in which medium composition is one of the key factors. In this perspective, the higher level of amylolysin synthesis obtained in complex medium emphasises the important role played by tryptone and yeast extract components in these mechanisms.

Activity of bacteriocin in food is greatly influenced by factors such as food composition, interaction with food components, sensitivity to meat protease, pH or storage temperature [12, 13]. Degradation within a few hours attributed to proteolytic activity has been demonstrated for different bacteriocins including pediocin AcH, nisin and sakacin P in different food matrices [1, 20]. By contrast to nisin, the stability of amylolysin during incubation with meat protease points to its low sensitivity to meat endogenous proteolytic content. The capacity of amylolysin to inhibit proliferation of different isolates of *L. monocytogenes* in poultry meat was demonstrated for an amylolysin concentration of 5–10 μg/g. This highlights the potent antilisterial activity of the bacteriocin. By contrast to nisin, which clearly showed a bactericidal effect, amylolysin had a bacteriostatic effect on *L. monocytogenes* under our experimental conditions.

This first characterisation demonstrated that amylolysin, a bacteriocin produced by *B. amyloliquefaciens* GA1, has a potent ability to protect poultry meat from the food-borne pathogens *L. monocytogenes*, suggesting its potential use as a biopreservative. However, further investigations on other food matrices and commercial foods and on the interaction of amylolysin with their constituents must be conducted.

Acknowledgments A. Arguelles-Arias is a PhD fellow of the FRIA (Fonds pour la Recherche dans l'Industrie et l'Agriculure, Belgium).

P. Fickers was a post-doctoral researcher at the FRS-FNRS (Fonds de la Recherche Scientifique, Belgium). The authors want to acknowledge Ch. Franz for critical reading of the manuscript.

References

1. Aasen I, Markussen S, Moretro T, Katla T, Axelsson L, Naterstad K (2003) Interactions of the bacteriocins sakacin P and nisin with food constituents. Int J Food Microbiol 87:35–43
2. Arguelles-Arias A, Ongena M, Halimi B, Lara Y, Brans A, Joris B, Fickers P (2009) *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens. Microb Cell Fact 8:63
3. Babasaki K, Takao T, Shimonishi Y, Kurahashi K (1985) Subtilosin A, a new antibiotic peptide produced by *Bacillus subtilis* 168: isolation, structural analysis, and biogenesis. J Biochem 98: 585–603
4. Bizani D, Dominguez AP, Brandelli A (2005) Purification and partial chemical characterization of the antimicrobial peptide cerein 8A. Lett Appl Microbiol 41:269–273
5. Carr F, Chill D, Maida N (2002) The lactic acid bacteria: a literature survey. Crit Rev Microbiol 28:281–370
6. Chatterjee S, Lad SJ, Phansalkar M, Rupp R, Ganguli B, Fehlhaber H, Kogler H (1992) Mersacidin, a new antibiotic from *Bacillus.* Fermentation, isolation, purification and chemical characterization. J Antibiot 45:832–838
7. Chen XH, Koumoutsi A, Scholz R, Schneider K, Vater J, Sussmuth R, Piel J, Borriss R (2009) Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. J Biotechnol 140:27–37
8. Cleveland J, Montville T, Nes I, Chikindas M (2001) Bacteriocins: safe, natural antimicrobials for food preservation. Int J Food Microbiol 71:1–20
9. Dortu C, Huch M, Holzapfel W, Franz CH, Thonart P (2008) Anti-listerial activity of bacteriocin-producing Lactobacillus curvatus CWBI-B28 and Lactobacillus sakei CWBI-B1365 on raw beef and poultry meat. Lett Appl Micobiol 47:581–586
10. Dortu C, Fickers P, Franz CH, Ndagano D, Huch M, Holzapfel W, Joris B, Thonart P (2009) Characterisation of an antilisterial bacteriocin produced by *Lactobacillus sake*i CWBI-B1365 isolated from raw poultry meat and determination of factors controlling its production. Prob Antimicrob Prot 1:75–84
11. Egan A (1983) Lactic acid bacteria of meat and meat products. Antonie Van Leeuwenhoek 49:327–336
12. Galvez A, Abriouel H, Lopez RL, Ben Omar N (2007) Bacteriocin-based strategies for food biopreservation. Int J Food Microbiol 120:51–70
13. Galvez A, Lopez RL, Abriouel H, Valdivia E, Omar NB (2008) Application of bacteriocins in the control of foodborne pathogenic and spoilage bacteria. Crit Rev Biotechnol 28:125–152
14. Gireesh T, Davidson B, Hillier A (1992) Conjugal transfer in *Lactococcus lactis* of a 68-kilobase-pair chromosomal fragment containing the structural gene for the peptide bacteriocin nisin. Appl Environ Microbiol 58:1670–1676
15. Gravesen A, Sorensen K, Aarestrup F, Knochel S (2001) Spontaneous nisin-resistant *Listeria monocytogenes* mutants with increased expression of a putative penicillin-binding protein and their sensitivity to various antibiotics. Microb Drug Resist 7: 127–135
16. Kiss A, Baliko G, Csorba A, Chuluunbaatar T, Medzihradszky K, Alfoldi L (2008) Cloning and characterization of the DNA region responsible for Megacin A-216 production in *Bacillus megaterium* 216. J Bacteriol 190:6448–6457
17. Klein C, Kaletta C, Entian K (1993) Biosynthesis of the lantibiotic subtilin is regulated by a histidine kinase/response regulator system. Appl Environ Microbiol 59:296–303
18. Liu W, Hansen J (1990) Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. Appl Environ Microbiol 56:2551–2558
19. Lubelski J, Rink R, Khusainov R, Moll G, Kuipers O (2008) Biosynthesis, immunity, regulation, mode of action and engineering of the model lantibiotic nisin. Cell Mol Life Sci 65: 455–476
20. Murray M, Richard J (1997) Comparative study of the antilisterial activity of nisin A and pediocin AcH in fresh ground pork stored aerobically at 4 °C. J Food Prot 60:1534–1540
21. Naghmouchi K, Kheadr E, Lacroix C, Fliss I (2007) Class I/Class IIa bacteriocin cross-resistance phenomenon in *Listeria monocytogenes*. Food Microbiol 24:718–727
22. Nissen-Meyer J, Rogne P, Oppegard C, Haugen H, Kristiansen P (2009) Structure-function relationships of the non-lanthionine-containing peptide (class II) bacteriocins produced by gram-positive bacteria. Curr Pharm Biotechnol 10:19–37
23. Pedersen P, Bjornvad M, Rasmussen M, Petersen J (2002) Cytotoxic potential of industrial strains of *Bacillus* sp. Regul Toxicol Pharmacol 36:155–161
24. Sutyak K, Wirawan R, Aroutcheva A, Chikindas M (2008) Isolation of the *Bacillus subtilis* antimicrobial peptide subtilosin from the dairy product-derived *Bacillus amyloliquefaciens*. J Appl Microbiol 104:1067–1074
25. Swaminathan B, Gerner-Smidt P (2007) The epidemiology of human listeriosis. Microbes Infect 9:1236–1243
26. Tsai HJ, Sandine W (1987) Conjugal transfer of nisin plasmid genes from *Streptococcus lactis* 7962 to Leuconostoc dextranicum 181. Appl Environ Microbiol 53:352–357