J Ind Microbiol Biotechnol (2007) 34:373–379
DOI 10.1007/s10295-007-0206-8

ORIGINAL PAPER

Mode of action of acidocin D20079, a bacteriocin produced by the potential probiotic strain, *Lactobacillus acidophilus* DSM 20079

Sahar F. Deraz · Eva Nordberg Karlsson · Ashraf A. Khalil · Bo Mattiasson

Received: 7 August 2006 / Accepted: 7 January 2007 / Published online: 26 January 2007

Abstract *Lactobacillus acidophilus* DSM 20079 is the producer of a novel bacteriocin termed acidocin D20079. In this paper, mode of action using three various concentrations of acidocin D20079 (2,048, 128 and 11.3 AU/ml) was determined against an indicator strain *L. delbrueckii* subsp. *lactis* DSM 20076. These concentrations all led to marked decreases in both the number of viable cells and in optical density, indicating that the activity of the acidocin D20079 was bactericidal with concomitant cell lysis. Moreover, the probiotic potential of *L. acidophilus* DSM 20079 was analyzed for its ability to survive and retain viability at conditions (acid and bile concentrations) mimicking the gastrointestinal (GI) tract, under which it survived exposure to pH 2.0 with a 1.2 log cycle reduction in viability and where 45% of the original population survived in a medium containing 0.3% bile for 3 h.

Keywords Acidocin D20079 · *Lactobacillus* · Bacteriocin · Antimicrobial protein · Inhibitory activity · Probiotic

S. F. Deraz · E. N. Karlsson · B. Mattiasson
Department of Biotechnology,
Center for Chemistry and Chemical Engineering,
Lund University, PO Box 124, 22100 Lund, Sweden

S. F. Deraz (✉) · A. A. Khalil
Department of Protein Technology,
Genetic Engineering and Biotechnology Institute,
Mubarak City for Science,
New Borg Alarab 21934, Alexandria, Egypt
e-mail: sahar_deraz@hotmail.com

Introduction

Development and optimization of the lactic acid bacteria (LAB) and their attendant 'natural' inhibitors as biopreservatives to control undesirable bacteria currently remains a primary focus of several laboratories involved in research concerning food safety and quality [1, 2]. Strategies utilized to study incorporation of biopreservatives into food include; (1) direct use of LAB-strains with proven antimicrobial activity as starter cultures or starter adjuncts (probiotic concept), (2) use of a biopreservative preparation in the form of previously fermented product, or (3) use of semipurified, purified, or chemically synthesized bacteriocins [3–5]. Today LAB are involved in the fermentation process of different kinds of food, such as dairy products, meat products, sour dough and other cereal products and various fermentable vegetables [6].The most common way to date, to develop LAB-fermented products with a functional food approach, has been to use probiotic LAB cultures. When selecting a probiotic strain, a number of aspects should be considered, and the theoretical basis for selection should involve safety, functional as well as technological aspects [7–9]. Safety aspects include the following specifications: strains for human use, should have a history of being non-pathogenic and have no association with diseases such as gastrointestinal (GI) disorders and this is the case with *Lactobacillus acidophilus* species.

Recent work in the field of probiotics have demonstrated an impressive increase in the interest of *L. acidophilus* as a probiotic agent, and have contributed to its application in functional foods and supplements in a worldwide market [10, 11]. *L. acidophilus* is a nonpathogenic and a member of the normal intestinal

microflora. It is widely used for production of fermented dairy products in the world. Furthermore, *L. acidophilus* is of considerable industrial and medical interest because this species is believed to play a role in human health and nutrition by its influence on the intestinal flora, where it is both reported to aid in the reduction of the levels of harmful bacteria and yeasts in the small intestine and to produce lactase, an enzyme which is important for the digestion of milk [12]. This effect was found to be both species and strain dependent.

Even though a potential probiotic strain fulfils the necessary safety criteria, the aspects related to production and processing are also of utmost importance. Several technological aspects therefore have to be considered in probiotic selection, such as, good sensory properties, viability during processing, stability in the product and during storage. Probiotic foods are currently restricted predominantly to fermented milk drinks and yogurt. The development of fermented, non-diary, oat-based product requires stringent selection of probiotic strains to maintain β-glucan level (the main active component for the cholesterol lowering properties recognized in oats), viability throughout processing and storage period till consumption.

In previous work [13], the survival of *L. acidophilus* DSM 20079 strain from human origin, during refrigerated storage in three oat-based products on a step in the development of new fermented oat-based non-dairy products was evaluated. The *L. acidophilus* DSM 20079 strain exhibited a viability of 10^6 cfu ml^{-1} after 30 days in the oat products during cold storage and no change of the β-glucan level was seen.

To fully cover all the theoretical bases for selecting probiotic strain, the functional requirements should be established by using in vitro methods. In order to exert probiotic effects in the human body, the strain has to be able to survive through the GI-tract (acid tolerance and tolerance to human gastric juice and bile tolerance). Also, ability of some *L. acidophilus* strains to produce bacteriocin could be an important property for a probiotic strain because a bacteriocin will probably help in the colonization of its producer in the GI tract. In previous study, [14] a novel natural antimicrobial agent produced by *L. acidophilus* DSM 20079 was isolated and named acidocin D20079. This peptide exhibited antimicrobial activity against a LAB-species, from which some strains are known to cause anaerobic spoilage of vacuum-packaged meat. In addition, acidocin D20079 exhibited extraordinary heat stability and can also work at a wide pH range. Since the probiotic effect can be different between strains belonging to the same species, the main objective of this study was hence to evaluate the functional requirements to cover all the theoretical bases for a strain to be used as probiotic. The survival of *L. acidophilus* DSM 20079 using conditions simulating those of GI-tract and the mode of action (bacteriocidal or bacteriostatic) of its bacteriocin was studied for further applications.

Materials and methods

Bacterial strains and media

The bacteriocin producer-strain *L. acidophilus* DSM 20079, and the sensitive indicator strain *L. delbrueckii* subsp. *lactis* DSM 20076 were obtained from DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The cultures used in this study were maintained as frozen stocks at –80°C and propagated twice (1% inoculum) in MRS broth (Difco Laboratories, Detroit, MI, USA) for 14–16 h at 37°C before experimental use. Agar plates were prepared by the addition of 1.5% (w/v) granulated agar (Difco Laboratories). Soft agar was prepared by adding 0.75% (w/v) agar to broth media. For bacteriocin production, a modified medium (DO-MRS), was used. The DO-MRS broth was prepared by dissolving all the ingredients of the MRS broth except Tween 80, which was replaced by oleic acid (Merck, Darmstadt, Germany) added to a final concentration of 0.1% (v/v). After adding all medium components, the mixture was autoclaved.

Bacteriocin production

Lactobacillus acidophilus DSM 20079 was propagated twice at 37°C (as described above) in MRS broth before inoculation in MRS [14] for bacteriocin production. A 3 l bioreactor (Chemofem FLC-B-3, Hägersten, Sweden) with a 2.5 l working volume of broth-medium was inoculated (1% inoculum) with the producer strain twice propagated in MRS medium. The cultivation temperature was maintained at 37°C and the culture was agitated at 150 rpm. The pH was 6.0 and was controlled by an automated pH-controller by titration with 12% ammonium hydroxide solution. Samples of 10 ml were removed for activity assays and optical density measurements. After 16–18 h, the culture pH was adjusted to pH 5.0 with hydrochloric acid, and at this point, cells were harvested and removed by centrifugation (4,000×*g*, 10 min) and stored at –20°C until bacteriocin analysis started.

Bacteriocin activity assay

The culture supernatants were assayed for bacteriocin activity by the spot on lawn technique with MRS agar using *L. delbrueckii* subsp. *lactis* DSM 20076 as indicator strain [15]. Indicator lawns were prepared by adding 0.125 ml of ten times diluted overnight culture to 5 ml of MRS soft agar (0.75%). The contents of the tubes were gently mixed and poured over the surfaces of pre-poured MRS agar plates. Bacteriocin samples were sterilized by passage through a 0.45 μm cellulose acetate filter.

Activity was estimated by the critical dilution method, using serial twofold dilutions in the same medium as used for the growth of the indicator strain. Activity was quantified by taking the reciprocal of the highest dilution that exhibited a clear zone of inhibition and was expressed as activity units (AU) per milliliter of culture medium. The titre of the bacteriocin solution, in AU/ml, was calculated as $(1000/d) \times D$, where D is the dilution factor and d is the dose (the amount of bacteriocin solution pipetted on each spot) [16].

Bacteriocin mode of action

Preparation of concentrated bacteriocin solution

The culture supernatant (400 ml) from cultivation in MRS was obtained as described above. After adjustment of the pH to 6.5 and treatment with catalase, the supernatant was precipitated with 40% ammonium sulfate for 18 h at 4°C. The precipitate was pelleted by centrifugation 10,000×*g* for 30 min and resuspended in 25 ml of 50 mM sodium phosphate buffer pH 7.0, then sterilized through 0.45 μm cellulose acetate filter. Activity was determined by the critical dilution method (described above).

Mode of action and adsorption of the bacteriocin to the sensitive bacterial cells

To investigate the mode of action of acidocin D20079, the indicator strain was grown at 37°C for 14–16 h in MRS. The cells were then washed and suspended in sterile 50 mM sodium phosphate buffer pH 7.0 to give ca. 10^8–10^9 cfu ml^{-1}. To this cell suspension, concentrated bacteriocin solution was added to final concentrations of 2,048, 128 and 11.3 AU/ml. As a control, a sample without bacteriocin activity was used.

Samples and controls of the same final volume were incubated at 37°C for 6 h. Optical density (615 nm), cell numbers (log cfu ml^{-1}) and bacteriocin activity (AU/ml) were determined at constant intervals [17]. The percentage of killing was calculated as follows:

$$\%\ \text{Killing} = [((\text{initial viable cells}) - (\text{final viable cells}))/(\text{initial viable cells})] \times 100.$$

Acid tolerance

Preparation of simulated gastric juice

Simulated gastric juice was prepared fresh, daily, by suspending pepsin (3 g l^{-1}) (P–7000, Sigma–Aldrich, St. Louis, MO, USA) in sterile saline (0.5% w/v) and adjusting the pH to 2.0 and 2.5 with concentrated HCl [18].

Preparation of washed cell suspensions and acid tolerance test

Lactobacillus acidophilus DSM 20079 was propagated twice in MRS broth, as described above. The cells from a 100 ml MRS culture were harvested by centrifugation (4,300×*g*, 10 min), and washed three times in phosphate-buffered saline, pH 7.0. Washed cell pellets, were then suspended in (1/10) × cultivation volume in the same buffer, hence obtaining a tenfold increase in cell density. To 1 ml of the washed cell suspension, 5 ml of simulated gastric juice and 1.5 ml NaCl (0.5% w/v) were added. The materials were vortexed for 10 s and incubated at 37°C for 3 h. Aliquots of 0.1 ml were then removed at constant intervals (0, 1, 2, 3 h) for determination of total viable count. Dilutions were made and cells were plated in duplicates on MRS agar (Difco). Plates were incubated at 37°C under anaerobic conditions using BBL anaerobic jars (BBL Micriobiological systems, Division of Beekton Dickinson and Co., Cockeysuille, MD, USA) for 72 h before enumeration.

Bile tolerance

Bile containing MRS broth was prepared by the addition of 0.1, 0.3, and 0.5% (v/v) of oxidized bile (Sigma–Aldrich, B-8381). The cells from a 100 ml 16 h MRS culture of *L. acidophilus* DSM 20079 were collected by centrifugation (3,400×*g*, 10 min), washed twice in saline (8.5 g NaCl/l) and resuspended in 10 ml MRS broth. This suspension was inoculated (1%) into MRS broth lacking or containing bile. After 0, 1, 2 and 3 h of incubation at 37°C, viable counts on MRS agar plates and optical density of the culture at 625 nm were determined [19].

Results

Mode of action and adsorption of the bacteriocin onto the sensitive bacterial cells

The effect of acidocin D20079 on the sensitive strain (*L. delbrueckii* subsp. *lactis* DSM 20076) was examined to establish whether it has a bactericidal or a bacteriostatic mode of action. A concentrated bacteriocin extract (broth from a *L. acidophilus* DSM 20079 cultivation in MRS, precipitated with ammonium sulfate (40%), and dissolved in phosphate buffer) with an initial activity level of 4,069 AU/ml was added to cell suspensions of the sensitive strain to final concentrations of 2,048, 128 and 11.3 AU/ml (Table 1).

These concentrations all led to marked decreases in both the number of viable cells and in optical density. In particular, as compared to controls without bacteriocin, the addition of the two highest concentrations (2,048 and 128 AU/ml of acidocin D20079 to the *L. delbrueckii* subsp. *lactis* DSM 20076 cells) resulted in high reductions of the respective cell population. These reductions corresponded to 95 and 94% of the viable cells, respectively, after 2 h, while at an acidocin concentration of 11.3 AU/ml, about 60% of the population was killed after the same time. After 4 h, 99.5, 98.5 and 85.5% of the population was killed by the bacteriocin concentrations of 2,046, 128 and 11.3 AU/ml, respectively (Table 1). A clear decrease in optical density of the cell suspension was also observed over time in the experiment with different bacteriocin concentrations, and was found to be concentration-dependent (Fig. 1), indicating that activity of the acidocin D20079 was bactericidal with concomitant cell lysis.

In addition, the residual bacteriocin activity was found to decrease from the initial value to very low or undetectable levels of activity after completing the 6 h incubation time, suggesting that the peptide was adsorbed.

Simulation tests of acid and bile tolerance indicates a probiotic potential of *Lactobacillus acidophilus* DSM 20079

In previous work, a novel bacteriocin (acidocin D20079) was isolated from the LAB strain *L. acidophilus* DSM 20079 [14]. The presence of this peptide indicated a potential of the strain (or its peptide) as an antimicrobial food additive. In order to analyse survival of the strain in the gut, simulation tests were performed. *L. acidophilus* DSM 20079 had a good viability in the simulated gastric juice experiments (which were all replicated twice). After 1 h at pH 2 and

Table 1 The mode of action of acidocin D20079

Time (h)	Control (cfu)	2,048 AU/ml				128 AU/ml				11.3 AU/ml			
		cfu	log	Killing (%)	RBA AU/ml	cfu	Log	Killing (%)	RBA AU/ml	cfu	log	Killing (%)	RBA AU/ml
0	2.9×10^8	2.6×10^8	8.4	0	2,048	2.6×10^8	8.4	0	128	2.7×10^8	8.4	0	11.3
2	2.9×10^8	1.3×10^7	7.1	95	64	1.4×10^7	7.1	94	56	1.1×10^8	8.0	59.2	0
4	2.5×10^8	1.2×10^6	6.1	99.5	11.3	3.3×10^6	6.5	98.7	0	3.9×10^7	7.5	85.5	0
6	2.7×10^8	6.3×10^5	5.8	99.7	2.83	1.9×10^6	6.2	99	0	8×10^6	6.9	97	0

The cfu of the indicator strain (*L. delbrueckii*) were calculated, after exposure to varying amounts of the bacteriocin

cfu Colony forming unite, *RBA* residual bacteriocin activity

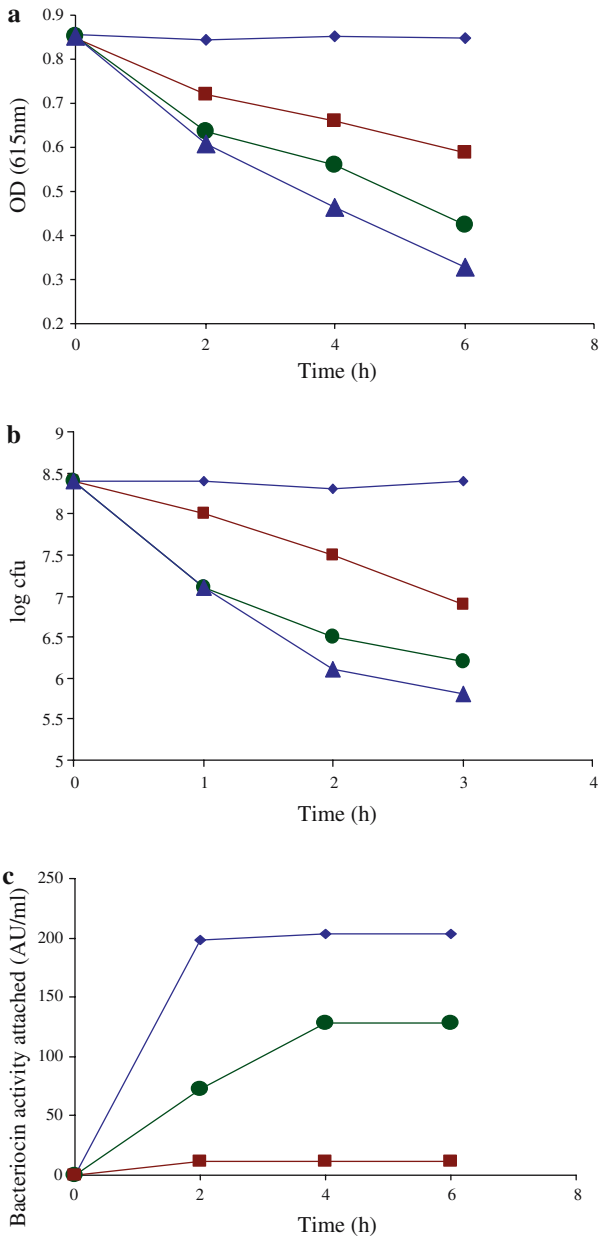

Fig. 1 Effect of acidocin D20079 on the indicator cells of *L. delbrueckii* subsp. *lactis* DSM 20076. Parameters tested were: optical density 615 nm (**a**), cell viability (**b**), and bacteriocin activity attached to indicator cells (**c**). *Filled triangle* 2,048 AU/ml, *filled circle* 128 AU/ml, *filled square* 11.3 AU/ml, *filled diamond* control

2.5, the number of viable cells had only slightly decreased to 96 and 98% of the initial viable count, at the respective pH, and the population size was 3.0×10^7 and 1.1×10^8 cfu ml^{-1}, respectively. After 3 h, a 1.2 and 1.0 log cycle reduction in viability was found at pH 2.0 and 2.5 with final populations of 3.5×10^6 and 1.4×10^7 cfu ml^{-1}, respectively.

A bile concentration of 0.3% is usually used for screening of bile resistant strains, as this is considered as an average intestinal bile concentration of the human gastrointestinal tract [20]. In this work, the ability of the strain to survive for 3 h was examined in the presence of bile in the concentration range of 0.1–0.5% w/v (Fig. 2). The lowest concentration (0.1%), did not significantly affect the survival. At 0.3 and 0.5% bile, a decrease in the number of viable cells was first observed after 1 h at 37°C. After completing the 3 h incubation, 45% of the original population survived in a medium with 0.3% bile, whereas at 0.5% bile only 5% survived the 3 h incubation. Based on these results *L. acidophilus* DSM 20079 was judged to exhibit an appreciable level of survival and was considered intrinsically tolerant to bile.

Discussion

Bacteriocins may possess a bacteriocidal or bacteriostatic mode of action on sensitive cells, this distinction being greatly influenced by several factors such as bacteriocin dose and degree of purification, physiological state of the indicator cells (e.g. growth phase) and experimental conditions (e.g. temperature, pH, presence of agents disrupting cell wall integrity and other antimicrobial compounds). Most bacteriocins exert bacteriocidal mode of action against the sensitive microorganisms, although some of the bacteriocins have been shown to act in a bacteriostatic manner. Bacteriocidal activity of bacteriocins may be accompanied by lysis of sensitive cells (bacteriolytic bacteriocins) [21], as shown for acidocin D20079, nisin A [22] and enterocin EFS2 [23]. The outer surface of Gram-negative bacteria contains lipopolysaccharides (LPS), and that of Gram-positive bacteria contains

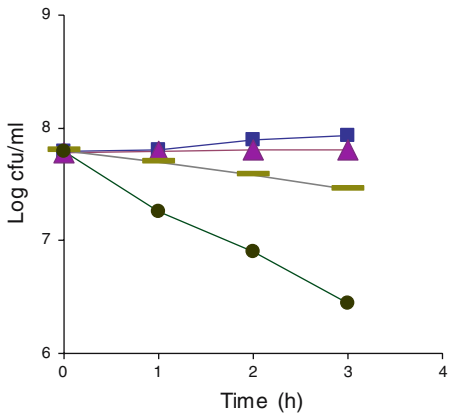

Fig. 2 The survival of *L. acidophilus* DSM 20079 in MRS broth with: 0% *filled square*, 0.1% *filled triangle*, 0.3% *thick line* and 0.5% *filled circle* (w/v) bile salts during incubation at 37°C, the reported values are the mean of two trials

acidic polysaccharids (teichoic acid), conferring a net negative charge to the surface of both Gram-negative and positive bacteria [24]. Binding of these bacteriocins to the negatively charged cell wall of the sensitive bacteria lead to release and therefore activation of autolytic enzymes, which under normal conditions are electrostatically bound to these polymers leading to lysis of the sensitive cells [22, 25]. Bacteriocins exert their bactericidal mode of action by destabilization and permeabilization of sensitive cell membranes [25].

In order to survive and establish within the human GIT, some of the desirable properties of probiotics include their ability to resist the acidity (pH 2.5–3.5) of the stomach [26] and the exposure to bile in the upper part of the intestine. In the present study, the exposure to pH 2.0 and 2.5 showed to be satisfactory. Although the pH in the stomach can be as low as pH 1.0, a high survival rate at pH 2.5 for at least 3 h, is often considered satisfactory, especially as probiotic strains can be buffered by food or other carrier molecules and in fact are not directly exposed to such a low pH in the stomach [27, 28]. It is recommended to use 0.3% bile as a proper concentration for selection of probiotic strains [20]. In our study, 45% (considered as an appreciable survival level) of the original population of *L. acidophilus* DSM 20079 resisted 0.3% bile.

Acknowledgments Mubarak City for Science and the Egyptian Ministry of Scientific Research are gratefully acknowledged for supporting the PhD project of Sahar Deraz ,and her stay at the Deparment of Biotechnology, Lund University. Special thanks for World Laboratory for additional support of the current study.

References

1. Eijsink VGH, Axelsson L, Diep DB, Havarstein LS, Holo H, Nes IF (2002) Production of class II bacteriocins by lactic acid bacteria, an example of biological warfare and communication. Antonie Van Leeuwenhoek 81:639–654
2. Chen H, Hoover DG (2003) Bacteriocins and their food applications. Compr Rev Food Sci Food Safety 2:82–100
3. De Vuyst L, Vandamme EJ (1994) Antimicrobial potential of lactic acid bacteria. In: De Vuyst L, Vandamme EJ (eds) Bacteriocins of lactic acid bacteria, microbiology, genetics and applications. Blackie Academic and Professional, Glasgow, pp 91–141
4. Montville TJ, Winkowski K (1997) Biologically based preservation systems and probiotic bacteria. In: Doyle MP, Beuchat LR, Montville TJ (eds) Food microbiology. Fundamentals and frontiers. ASM press, USA, pp 557–576
5. Luchansky JB (1999) Overview in applications for bacteriocin-producing lactic acid bacteria and their bacteriocins. Antonie Van Leeuwenhoek 76:335–335
6. Hammes WP, Weiss N, Holzapfel W (1991) The genera *Lactobacillus* and *Carnobacterium*. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes, vol II, 2nd edn. Springer, Heidelberg, pp 1535–1594
7. Salminen S, Deighton MA, Benno Y, Gorbach SL (1998) Lactic acid bacteria in health and disease. In: Salminen S, Von Wright A (eds) Lactic acid bacteria: microbiology and functional aspects, 2nd edn. Marcel Dekker Inc, New York, pp 211–253
8. Adams MR (1999) Safety of industrial lactic acid bacteria. J Biotechnol 68:171–178
9. Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T (2000) Probiotic bacteria: safety, functional and technological properties. J Biotechnol 84:197–215
10. Naidu AS, Bidlack WR, Clemens RA (1999) Probiotic spectra of lactic acid bacteia. Crit Rev Food Sci Technol 38:13–126
11. Kitazawa H, Ino T, Kawai Y, Itoh T, Saito T (2002) A novel immunostimulating aspect of *Lactobacillus gasseri*: induction of 'Gasserokine' as chemoattractants for macrophages. Int J Food Microbiol 77:29–38
12. Kaur IP, Chopra K, Saini A (2002) Probiotics: potential pharmaceutical applications. Eur J Pharm Sci 15:1–9
13. Martensson O, Oste R, Holst O (2002) The effect of yoghurt culture on the survival of probiotic bacteria in oat-based, non dairy products. Food Res Int 35:775–784
14. Deraz S, Karlsson NE, Hedström M, Andersson MM, Mattiasson B (2005) Purification and characterisation of acidocin D20079, a bacteriocin produced by *Lactobacillus acidophilus* DSM20079. J Biotechnol 117:343–354
15. Barefoot SF, Klenhammer TR (1983) Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl Environ Microbiol 45:1808–1815
16. Parente E, Brienza C, Moles M, Ricciardi A (1994) A comparison of methods for the measurement of bacteriocin activity. J Microbiol Meth 22:95–108
17. Mataragas M, Metaxopoulos J, Drosinos HE (2002) Characterization of two bacteriocins produced by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L 442, isolated from dry fermented sausages. World J Microbiol Biotechnol 18:847–856
18. Charteris PW, Kelly MP, Morelli L, Collins KJ (1998) Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. J Appl Microbiol 84:759–768
19. Matijasic BB, Rogelj I (2000) *Lactobacillus K7*—a new candidate for a probiotic strain. Food Technol Biotechnol 38:113–119
20. Gilliland ES, Stalely ET, Bush JL (1984) Importance of bile tolerance of *Lactobacillus acidophilus* used as dietary adjunct. J Dairy Sci 67:3045–3051
21. Cintas LM, Casaus MP, Herranz C, Nes IF, Hernandez PE (2001) Bacteriocins of lactic acid bacteria. Food Sci Technol Int 7:281–305
22. Bierbaum G, Sahl HG (1991) Induction of autolysis *Staphylococcus simulans* 22 by Pep 5 and nisin and influence of the cationic peptides on the activity of the autolytic enzymes. In: Jung G, Sahl HG (eds) Nisin and novel lantibiotics. Escom Publishers, Leiden, pp. 386–396
23. Maisnier-Patin S, Forni E, Richard J (1996) Purification, partial characterization and mode of action of enterococcin EFS2, an antilisterial bacteriocin produced by a strain of *Enterococcus faecalis* isolated from cheese. Int J Food Microbiol 30:255–270
24. Brock TD (1974) Biology of microorganisms, 2nd edn. Prentice-Hall, Englewood Cliffs
25. Jack RW, Tagg JR, Ray B (1995) Bacteriocins of gram-positive bacteria. Microbiol Rev 59:171–200

26. Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JHJ (1998) Overview of gut flora and probiotics. Int J Food Microbiol 41:85–101
27. Conway LP, Gorbach LS, Goldin RB (1987) Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J Dairy Sci 70:1–12
28. Prasad J, Gill H, Smart J, Gopal KP (1998) Selection and characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotics. Int Dairy J 8:993–1002