RESEARCH ARTICLE

Fate and efficacy of lacticin 3147-producing *Lactococcus lactis* in the mammalian gastrointestinal tract

Alleson Dobson[1,2], Fiona Crispie[1,2], Mary C. Rea[1,2], Orla O'Sullivan[1], Pat G. Casey[2], Peadar G. Lawlor[3], Paul D. Cotter[1,2], Paul Ross[1,2], Gillian E. Gardiner[4] & Colin Hill[2]

[1]Teagasc, Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland; [2]Alimentary Pharmabiotic Centre, University College Cork, Ireland; [3]Teagasc, Pig Development Department, Animal & Grassland Research & Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; and [4]Department of Chemical & Life Sciences, Waterford Institute of Technology, Waterford, Ireland

Correspondence: R. Paul Ross, Teagasc, Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland. Tel.: +353 254 2229; fax: +353 254 2340; e-mail: paul.ross@teagasc.ie

Received 2 July 2010; revised 17 January 2011; accepted 26 January 2011.
Final version published online 7 March 2011.

DOI:10.1111/j.1574-6941.2011.01069.x

Editor: Julian Marchesi

Keywords
bacteriocin; gastrointestinal tract; lacticin 3147; colon model.

Abstract

Gastrointestinal survival of the bacteriocin-producing strain, *Lactococcus lactis* DPC6520, was evaluated systematically *in vitro* and *in vivo* with a view to using this strain to deliver biologically active lacticin 3147, a broad-spectrum bacteriocin, to the gut. The activity of the lacticin 3147 producer was also evaluated against two clinically relevant pathogens: *Clostridium difficile* and *Listeria monocytogenes*. When suspended in an appropriate matrix, the lactococcal strain is capable of surviving simulated gastrointestinal juices similar to the porcine probiotic, *Lactobacillus salivarius* DPC6005. Upon administration of *L. lactis* DPC6520 to pigs ($n = 4$), excretion rates of $\sim 10^2$–10^5 CFU g^{-1} faeces were observed by day 5. Although passage through the gastrointestinal tract (GIT) did not affect lacticin 3147 production by *L. lactis* DPC6520 isolates, activity was undetectable in faecal samples by an agar well diffusion assay. Furthermore, *L. lactis* DPC6520 had no inhibitory effect on *C. difficile* or other bacterial populations in a human distal colon model, while lactococcal counts declined 10 000-fold over 24 h. The lacticin 3147 producer failed to prevent *L. monocytogenes* infection in a mouse model, even though a mean *L. lactis* DPC6520 count of 4.7×10^4 CFU g^{-1} faeces was obtained over the 5-day administration period. These data demonstrate that *L. lactis* DPC6520 is capable of surviving transit through the GIT, and yet lacks antimicrobial efficacy in the models of infection used.

Introduction

Bacteriocins are small, ribosomally synthesized, antimicrobial peptides produced by both Gram-positive and -negative microorganisms (Klaenhammer, 1993). Bacteriocins derived from lactic acid bacteria (LAB) in particular have a wide variety of potential applications as, for example, food biopreservatives and perhaps, more interestingly, as alternatives to antibiotics for medical and veterinary use (Cotter *et al.*, 2005; Klostermann *et al.*, 2010). Recent studies demonstrated that orally administered bacteriocin-producing *Lactobacillus*, *Pediococcus* and *Lactococcus* strains reduce intestinal infection *in vivo*, providing direct evidence of the clinical efficacy of bacteriocin-producing strains in the gastrointestinal tract (GIT; Corr *et al.*, 2007; Millette *et al.*, 2008). However, given the overall level of diversity associated with LAB bacteriocins, it should be noted that the reverse has also been reported (Stern *et al.*, 2008; Dabour *et al.*, 2009). The production of bacteriocins has long been considered a probiotic trait; probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (Pineiro & Stanton, 2007). Therefore, in this case, bacteriocins may not only reduce enteric infection but may also contribute to intestinal survival of probiotic strains by providing a competitive advantage over resident microbiota (Walsh *et al.*, 2008).

Lacticin 3147 is a two-component broad-spectrum bacteriocin produced by the food starter bacterium, *Lactococcus lactis* (Ryan *et al.*, 1996; Martin *et al.*, 2004; Morgan *et al.*, 2005). Concentrated preparations of lacticin 3147 were previously shown to inhibit clinically relevant Gram-

FEMS MICROBIOLOGY ECOLOGY

positive pathogens including *Streptococcus mutans* and *Clostridium difficile* (O'Connor *et al.*, 2006; Rea *et al.*, 2007). However, orally administered lacticin 3147 is rapidly degraded in the mammalian GIT (Gardiner *et al.*, 2007). An alternative strategy for targeted delivery of lacticin 3147 to the gut could be oral administration of the producing organism *L. lactis*. In this respect, studies from our laboratory have previously demonstrated the efficacy of lacticin 3147-producing cultures against such pathogens as *Listeria monocytogenes* and *Clostridium perfringens* in cheese and meat products, respectively (Scannell *et al.*, 2000; O'Sullivan *et al.*, 2006). However, as members of the genus *Lactococcus* are not normally considered part of the commensal microbiota, their ability to survive gastrointestinal transit and subsequent bacteriocin production *in vivo* is questionable.

The aim of this study was firstly to investigate the intestinal transit tolerance of lacticin 3147-producing *L. lactis* (DPC6520) initially *in vitro* using simulated intestinal juices and subsequently *in vivo* using both murine and porcine models. Secondly, the study evaluated the efficacy of lacticin 3147-producing *L. lactis* in controlling infection by two clinically relevant pathogens: *C. difficile* and *L. monocytogenes*.

Materials and methods

Bacterial strains and culture conditions

The isogenic lactococcal strains used in this study were generated as follows: *L. lactis* DPC3147, a lacticin 3147 producer, was cured of the lacticin 3147-producing plasmid, pMRC01, creating the strain *L. lactis* DPC5399 (D.P. Twomey *et al.*, pers. commun.). To facilitate subsequent enumeration in the GIT, a streptomycin-resistant derivative of *L. lactis* DPC5399 (designated *L. lactis* DPC6519) was created by selecting for resistance on streptomycin-containing medium. Molecular fingerprinting by randomly amplified polymorphic DNA (RAPD) PCR was used to confirm that the streptomycin-resistant variant was identical to the parent strain. The plasmid pMRC01 (which encodes all the genetic factors responsible for lacticin 3147 production and immunity) was then reintroduced into *L. lactis* DPC6519 by conjugation, creating the lacticin 3147-producing, streptomycin-resistant derivative, *L. lactis* DPC6520. The presence/absence of pMRC01 was confirmed by plasmid profile analysis, as outlined previously (Anderson & McKay, 1983), and lacticin 3147 production was confirmed using an agar well diffusion assay (Ryan *et al.*, 1996). *Lactococcus lactis* ssp. *cremoris* HP was used as an indicator in routine agar well diffusion antimicrobial activity assays; *L. lactis* ssp. *cremoris* HP (pMRC01), a lacticin 3147-insensitive derivative of *L. lactis* HP, was also used as an indicator in order to confirm that inhibition of the target strain was solely due to the production of lacticin 3147. *Lactobacillus salivarius* DPC6005, a porcine probiotic, was used as a positive control for all *in vitro* analyses. All lactococcal strains were routinely grown in M17 (Difco Laboratories; Detroit, MI) supplemented with 0.5% lactose (LM17) under aerobic conditions at 30 °C. *Lactobacillus salivarius* DPC6005 was routinely grown in de Man Rogosa Sharpe (MRS; Difco) anaerobically at 37 °C. *Clostridium difficile* ribotype 001 was routinely grown in reinforced clostridial medium (RCM; Difco) anaerobically at 37 °C. *Listeria monocytogenes* EGDe was grown in brain–heart infusion broth (BHI; Difco) and shaken aerobically at 37 °C.

Molecular fingerprinting

Pulsed-field gel electrophoresis (PFGE) or RAPD PCR analyses were performed on each administered *Lactococcus* strain as well as on faecal isolates from each animal trial. PFGE analyses were performed as described previously (Simpson *et al.*, 2002) using SmaI restriction endonuclease and a low-range molecular weight DNA marker (9.42–194.0 kb; New England Biolabs, Hitchin, Herts). RAPD PCR analyses were performed as outlined previously (Gardiner *et al.*, 2004).

In vitro gastric and ileum juice assays

Simulated gastric juice and ileum juice were prepared according to the method outlined previously (Beumer *et al.*, 1992), but with lipase omitted from the ileum juice. Cells from overnight cultures of *L. lactis* DPC6519, *L. lactis* DPC6520 and *L. salivarius* DPC6005 were harvested by centrifugation at 6000 ***g*** for 15 min, washed in maximum recovery diluent (MRD; Oxoid Ltd, Basingstoke, Hampshire, UK) and resuspended in one-tenth the initial volume in either MRD or 10% (w/v) reconstituted skim milk (RSM). Each bacterial cell suspension ($\geq 10^9$ CFU mL^{-1}) was added (1 : 1) to simulated gastric juice (pH 2.2) or simulated ileum juice (pH 7.0) and incubated at 37 °C with mild agitation for 2 and 5 h, respectively. Samples were taken at regular intervals and culture viability was determined as follows: samples were serially diluted 1 in 10 in MRD and appropriate dilutions were pour-plated on LM17 and MRS agars for lactococci and lactobacilli, respectively. LM17 plates were incubated aerobically at 30 °C for 2 days, while MRS plates were incubated anaerobically at 37 °C for 2 days. Each experiment was performed in duplicate with separate overnight cultures of each strain.

Pig-feeding trial

A pig-feeding trial was conducted to investigate the survival of *L. lactis* DPC6520 in the porcine gut. The trial complied with European Union Council Directive 91/630/EEC, which

outlines the minimum standards for the protection of pigs and European Union Council Directive 98/58/EC, which concerns the protection of animals kept for farming purposes. To prepare a culture suspension for administration to pigs, an overnight culture of the lacticin 3147-producing strain, *L. lactis* DPC6520, was first inoculated (1% v/v) into 5 L sterile LM17 broth and incubated overnight aerobically at 30 °C. Cells were harvested by centrifugation at 6000 ***g*** for 15 min, washed twice in MRD, resuspended in 500 mL 10% RSM and stored at 4 °C until administered to pigs (a maximum of 2 h). In this way, fresh culture suspensions containing $\sim 10^9$ CFU mL^{-1} *L. lactis* DPC6520 were prepared daily for the duration of the trial. To ensure consistency, bacterial counts in 10% RSM suspensions were periodically determined by plate counting on LM17 agar containing 1 mg mL^{-1} of streptomycin (Sigma-Aldrich Ireland Ltd., Dublin, Ireland).

Weaned pigs (Large White × Landrace, 4 weeks of age) were trained to drink 10% RSM during a 4-day acclimatization period. Eight pigs that were drinking RSM well were used in the feeding trial. The eight pigs were from two litters and were blocked on sex and weight before being randomly allocated to one of two groups ($n = 4$): a control group and a culture-fed group. Each animal was penned individually with control pigs penned in isolation from culture-fed pigs to prevent any cross-contamination. During the 5-day trial period, 100 mL of 10% RSM culture suspension containing $\sim 10^9$ CFU mL^{-1} *L. lactis* DPC6520 (prepared as outlined above) was administered daily to the culture-fed group, providing a total daily intake of $\sim 10^{11}$ CFU. This dose was chosen based on a preliminary pig trial that had shown that 10^9 CFU day^{-1} was ineffective. Control pigs received 100 mL sterile 10% RSM daily. In addition to the culture suspension or 10% RSM, animals also had unrestricted access to nonmedicated creep feed (manufactured in the Moorepark feed mill) and water for the duration of the 5-day trial. Faecal samples were collected by rectal stimulation from each pig on days 0 and 5, where day 0 was the day before the commencement of culture suspension/10% RSM administration. Faecal samples were stored at 4 °C and analysed within 2 h of collection to enumerate *L. lactis* DPC6520 as follows: samples were homogenized as 10-fold serial dilutions in a stomacher, further 10-fold dilutions were then prepared and appropriate dilutions were spread-plated onto lacticin 3147-LM17 plates prepared as described previously (Mills *et al.*, 2002) containing nystatin (50 U mL^{-1}; Sigma-Aldrich Ireland Ltd.) and streptomycin (1 mg mL^{-1}). Plates were incubated aerobically for 2 days at 30 °C. Representative colonies were randomly selected from day 5 faecal samples and analysed by PFGE, as outlined above, and by PCR amplification using *ltnαβ* primers (Fallico *et al.*, 2009) to determine whether they were the administered strain. The ability of *L. lactis* DPC6520 to produce lacticin 3147 after passage through the GIT was also evaluated using the agar well diffusion assay method. Additionally, lacticin 3147 activity was evaluated in the homogenized faecal samples by an agar well diffusion assay with the following modifications: 20 mL of sterile LM17 containing 1.5% (w/v) agar was seeded with 100 μL of the appropriate indicator strain and poured into a sterile petri dish. Once solidified, wells of uniform diameter were then bored into the medium and 50 μL of the homogenized faecal sample was then added to each well. Plates were incubated overnight aerobically at 30 °C and examined for zones of clearing.

Human distal colon model studies

To assess the performance of *L. lactis* DPC6520 in the human colon environment and to evaluate its efficacy against the noninvasive pathogen, *C. difficile*, a human distal colon model, was used. The experiment was conducted in duplicate using faecal samples from the same human donor. Firstly, a 20% (w/v) faecal slurry was prepared, whereby a fresh faecal sample from a healthy adult (who had not been prescribed antibiotics in the previous 3 months) was homogenized with 100 mM sodium phosphate buffer (pH 7.0) supplemented with 0.05% cysteine. Three sterile 300-mL fermentation vessels (Multifors, Infors HT; Bottmingen, Switzerland) containing 160 mL of sterile fermentation medium (Fooks & Gibson, 2003) were each inoculated with 35 mL of faecal slurry. The vessels were then sparged with O_2-free N_2 for approximately 1 h in order to generate anaerobic conditions. An overnight culture of *C. difficile* grown in RCM broth was then added to each vessel in order to obtain a final concentration of $\sim 10^6$ CFU mL^{-1}. Vessel A served as the control vessel and contained *C. difficile* only; vessel B was inoculated with *L. lactis* DPC6519, the lacticin 3147 nonproducer, while vessel C was inoculated with *L. lactis* DPC6520, the lacticin 3147-producer. To prepare *L. lactis* cultures, cells from 2 L overnight cultures of *L. lactis* DPC6519 and DPC6520 were harvested by centrifugation at 6000 ***g*** for 15 min, washed once in MRD and then resuspended in 2 mL MRD. *Lactococcus lactis* in these cell suspensions were enumerated on LM17 agar plates containing 500 μg mL^{-1} streptomycin before the experiment and volumes were adjusted accordingly in order to obtain equal concentrations of lactococci ($\sim 10^9$ CFU mL^{-1}) in each vessel. Fermentations were performed over a 24-h period at 37 °C, maintained at a constant pH of 6.8 by the automatic addition of 2 N NaOH, sparged with oxygen-free N_2 and with continuous stirring at 100 r.p.m. Samples were withdrawn from each of the three fermenters at regular intervals for microbiological analyses. *Clostridium difficile* was enumerated on Brazier's cycloserine cefoxitin egg yolk agar (LabM Ltd, Bury, Lancashire, UK) incubated anaerobically at 37 °C for 2 days. *Lactococcus lactis* DPC6519 was enumerated on LM17 agar supplemented with

streptomycin (500 μg mL^{-1}) and nystatin (50 U mL^{-1}) incubated aerobically at 30 °C for 2 days. *Lactococcus lactis* DPC6520 was enumerated on lacticin 3147-LM17 plates also containing streptomycin (500 μg mL^{-1}) and nystatin (50 U mL^{-1}) and incubated aerobically at 30 °C for 2 days. Lacticin 3147 activity from faecal slurry samples was evaluated using the agar well diffusion method. Samples were also analysed for the presence of lacticin 3147 peptides by matrix-assisted laser desorption ionization–time-of-flight MS (MALDI-TOF MS) (Cotter *et al.*, 2006). Briefly, 2 mL faecal slurry was centrifuged at 20 000 ***g*** for 2 min. The supernatant was then applied to a Strata C18E solid-phase extraction column (Phenomenex, Cheshire, UK) pre-equilibrated with 1 mL methanol. The column was subsequently washed with 40% ethanol and eluted using 70% isopropan-2-ol/0.1% trifluoroacetic acid. Purified lacticin 3147, prepared as outlined by Cotter *et al.* (2006), added to the faecal slurry from the control vessel, served as a spiked control.

High-throughput compositional sequencing

The effect of lacticin 3147-producing *L. lactis* on the colonic microbiota was also investigated using a pyrosequencing-based strategy. Duplicate samples were collected from each fermentation vessel at 24 h from each of two independent fermentation experiments and frozen at −20 °C upon collection. DNA extraction was performed on all of these samples using the QIAamp DNA Stool Mini Kit (Qiagen, West Sussex, UK). DNA from duplicate samples from each experiment was pooled, yielding two DNA samples for each vessel, one from each of the two independent experiments. PCR amplification was then performed in duplicate on each pooled DNA sample. Universal 16S primers were designed to amplify from highly conserved regions of the 16S rRNA gene corresponding to those flanking the V4 region. The forward primer F1 (5′-AYTGGGYDTAAAGNG) and a combination of four reverse primers R1 (5′-TACCRGGGTHTCTAATCC), R2 (5′-TACCAGAGTATCTAATTC), R3 (5′-CTACDSRGGTMTCTAATC) and R4 (5′-TACNVGGGTATCTAATC) (RDP's Pyrosequencing Pipeline: http://pyro.cme.msu.edu/pyro/help.jsp) were used for Taq-based PCR amplification. Amplicons were cleaned using the Agencourt AMPure purification system (Beckman Coulter Genomics, Danvers, MA), and sequenced in duplicate on a 454 Genome Sequencer FLX Titanium platform (Roche Diagnostics Ltd, West Sussex, UK) according to 454 protocols.

Raw sequencing reads were quality trimmed using a locally installed version of the RDP Pyrosequencing Pipeline. Clustering of the sequence data was performed using the MOTHUR software package (Schloss & Handelsman, 2008). The resulting BLAST output (Altschul *et al.*, 1997) using trimmed FASTA sequences was parsed using MEGAN (Huson *et al.*, 2007). Bit scores within MEGAN were used for filtering the results before tree construction and summarization; a bit score of 86 was selected as the cut-off as used previously (Urich *et al.*, 2008). The results are presented as the means of the data from the two independent experiments.

Murine *Listeria* infection studies

To assess the survival of *L. lactis* DPC6520 in the murine gut and to evaluate its ability to confer protection against *L. monocytogenes* infection, a mouse trial was performed. To prepare *L. lactis* culture suspensions for administration to mice, overnight cultures of *L. lactis* DPC6519 (lacticin 3147 nonproducer) and *L. lactis* DPC6520 (lacticin 3147 producer) were first inoculated (1% v/v) into 1 L of sterile LM17 and incubated aerobically overnight at 30 °C. Cells were harvested by centrifugation at 6000 ***g*** for 15 min, washed in sterile MRD and then diluted to 10^9 CFU mL^{-1} using sterile-phosphate-buffered saline (PBS). *Lactococcus* numbers were enumerated daily on LM17 agar supplemented with 500 μg mL^{-1} streptomycin in order to ensure consistency over the duration of the trial.

The mouse trial was performed according to the guidelines for the care and use of laboratory animals approved by the Irish government's Department of Health and Children. Harlan A/J mice (aged 7 weeks) were purchased from Harlan Ltd (Bicester, UK) and housed in a dedicated facility under standard conditions. Mice were fed with food and water unless otherwise stated. Before the start of the trial, mice were divided into three separate groups ($n = 5$): (1) a control group receiving PBS only, (2) a nonlacticin 3147 group consisting of mice administered a daily dose of $\sim 10^9$ CFU of the nonlacticin 3147-producing strain, *L. lactis* DPC6519 and (3) a lacticin 3147 group comprising mice fed a daily dose of $\sim 10^9$ CFU of the lacticin 3147-producing strain, *L. lactis* DPC6520. PBS or culture suspensions were administered once daily by oral pipette for the duration of the 5-day trial. Each group of mice was housed separately for the duration of the trial. Following 3 days of lactococcal culture or PBS administration, mice were challenged by oral pipette with $\sim 10^7$ CFU *L. monocytogenes* EGDe per mouse. Faecal samples were collected daily from each animal to determine lactococcal survival. Each sample was stored at 4 °C and analysed within 2 h. Samples were homogenized in MRD as 10-fold dilutions, further serially diluted in MRD and spread-plated onto either LM17 plates or lacticin 3147-LM17 plates to enumerate *L. lactis* DPC6519 or *L. lactis* DPC6520, respectively. All plates were supplemented with nystatin (50 U mL^{-1}) and streptomycin (500 μg mL^{-1}) and were incubated at 30 °C aerobically for 2 days. On the final day of the trial (day 5), mice were sacrificed by cervical dislocation and the caeca, livers and spleens were excised and homogenized in sterile MRD. Serial 10-fold dilutions

were prepared from these homogenates and appropriate dilutions were spread-plated on BHI agar incubated at 37 °C overnight to enumerate *L. monocytogenes*. Lacticin 3147 activity in murine faecal samples was evaluated using the agar well diffusion assay, as described above.

Statistical analyses

All microbiological data were $\log_{10}$-transformed before statistical analysis. *Clostridium difficile* and *L. lactis* counts from the simulated human distal colon model were analysed for repeated measures using the PROC MIXED procedure of STATISTICAL ANALYSIS SYSTEMS (SAS; SAS Institute Inc, Cary, NC) with individual fermentation vessels as the experimental unit. Likewise, faecal *L. lactis* counts from mice were analysed for repeated measures using the PROC MIXED procedure of SAS. In this case, the individual mouse was the experimental unit. The general linear model procedure of SAS was used for the analysis of caecal, liver and spleen *Listeria* counts and the individual mouse was the experimental unit.

Results and discussion

Because the overall goal of this study was to determine the suitability of a lacticin 3147-producing strain of *L. lactis* as a vehicle for delivery of bacteriocin to the GIT, a study of the survival of lacticin 3147-producing lactococci in the mammalian GIT and their performance against both invasive and noninvasive pathogens was undertaken.

Survival of lactococci in simulated intestinal conditions

It is known that a number of *L. lactis* strains are capable of surviving transit through the mammalian GIT, although the survival rates are variable depending not only on the strain used but also on the method of administration. Reported survival rates range from 0.1% to 40% (Klijn *et al.*, 1995; Drouault *et al.*, 1999; Kimoto *et al.*, 2003). Therefore, the potential of lacticin 3147-producing (*L. lactis* DPC6520) and -nonproducing (*L. lactis* DPC6519) lactococci to withstand gastric transit was first investigated in simulated gastric juice (pH 2.2) over a period of 2 h. Previous studies have indicated that over 80% of the stomach contents are emptied approximately 90 min after ingestion (Berrada *et al.*, 1991). When suspended in MRD, lactococcal counts declined from 1×10^9 to 9.1×10^3 CFU mL^{-1} for *L. lactis* DPC6519 and from 9.7×10^8 to 2×10^4 CFU mL^{-1} for *L. lactis* DPC6520 over a 1-h period (Fig. 1a). After a 2-h incubation, the survival of *L. lactis* DPC6519 declined to just 6×10^1 CFU mL^{-1} and to 4.5×10^2 CFU mL^{-1} for *L. lactis* DPC6520 (Fig. 1a). In contrast, the viability of *L. salivarius* DPC6005, a porcine probiotic known to survive intestinal

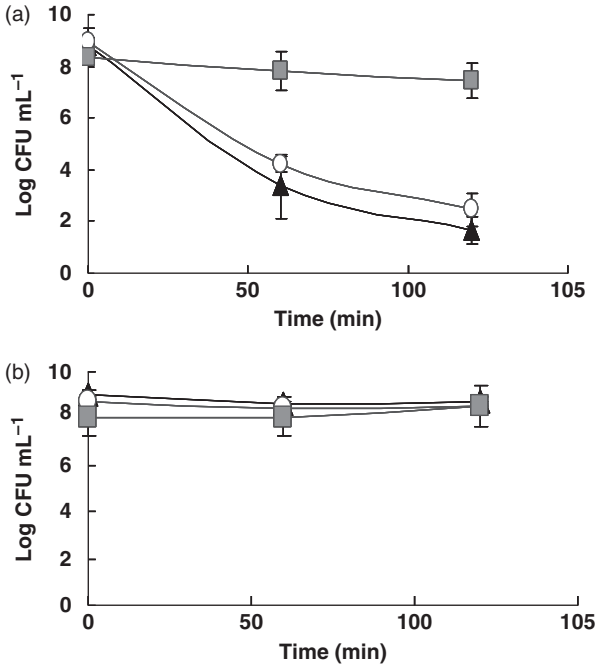

Fig. 1. Survival of *Lactococcus lactis* DPC6519 (▲), *L. lactis* DPC6520 (○) and *Lactobacillus salivarius* DPC6005 (■) in simulated gastric juice, pH 2.2, over time when suspended in MRD (a) or 10% RSM (b). Values are means of data from duplicate experiments, with SD indicated by vertical bars.

transit, remained relatively stable, decreasing only slightly from 5.2×10^8 to 4.7×10^8 CFU mL^{-1} in the same 2-h period. These findings are in agreement with a recent study, which found that strains of *L. lactis* did not tolerate gastric transit simulation as well as *Pediococcus* and *Lactobacillus* strains analysed (Millette *et al.*, 2008). However, when *L. lactis* strains were suspended in 10% RSM (Fig. 1b), a likely matrix in which cells may be ingested, both *L. lactis* DPC6519 and DPC6520 remained viable, with counts decreasing by only 0.3- and 0.2-$\log_{10}$, respectively, over a 2-h period. Survival rates were comparable to that seen for the *L. salivarius* probiotic (Fig. 1b).

The enhanced survival of probiotics in gastric juice using food components such as cereal extracts (Charalampopoulos *et al.*, 2003) cheese and yogurt (Gardiner *et al.*, 1999), and milk (Conway *et al.*, 1987) has been well characterized. Indeed, a previous study found that *L. lactis* JIM4886 cells are quite sensitive to gastric acidity in rats, and yet become relatively resistant when administered with food (Drouault *et al.*, 1999). The buffering capacity of the food matrix is most likely responsible for improved survival. Indeed, when skim milk-suspended cells were added 1 : 1 to simulated gastric juice in the present study, the pH increased from 2.2 to 5.3. As LAB species (including *Lactococcus* sp.) are innately acid tolerant, these conditions would be suitable for the survival of lactococci and other LAB (Klaenhammer

et al., 2002). In contrast, the addition of MRD-suspended cells in the same ratio led to a relatively small increase in pH (up to 2.6 only). However, it should be noted that the high pH resulting from ingestion of milk is not likely to be maintained under physiological conditions, as gastric juice would be continually secreted into the stomach *in vivo*.

Gardiner *et al*. (2007) demonstrated previously that lacticin 3147 is completely inactivated in porcine ileal digesta after 5 h. Therefore, *L. lactis* DPC6519 and DPC6520 were also exposed to simulated ileum juice. Following a 5-h exposure period, viability decreased by ~9-logs for both *L. lactis* DPC6519 and DPC6520 (Fig. 2a). In contrast, *L. salivarius* DPC6005 counts decreased by only 4-logs. However, viability was markedly improved when RSM-suspended cells were exposed to simulated ileum juice, with numbers of *L. lactis* DPC6519 and DPC6520 decreasing from the initial counts of 4.6×10^9 to 1.6×10^5 CFU mL^{-1} for *L. lactis* DPC6519 and from 9.5×10^9 to 7.5×10^4 CFU mL^{-1} for *L. lactis* DPC6520 during the 5-h exposure period (Fig. 2b). Survival of the probiotic control strain, *L. salivarius*, however, was still superior, with only a 1-log reduction (from 5.3×10^9 to 7.1×10^8 CFU mL^{-1}) observed in 5 h. Interestingly, as in the simulated gastric juice experiment, the survival of lactococci in the simulated ileum environment was enhanced in the presence of 10% RSM. Similar findings were reported by Ganzle *et al*. (1999), who showed that the survival of a bacteriocin-producing strain of *Lactobacillus curvatus* was improved when added together with meat to a simulated small intestine environment. Likewise, another study using *L. lactis* JIM4886 found an increase in lactococcal survival (from 0.01% to 30%) in the duodenum of rats if mixed with food, although this effect was dependent on the dose of *L. lactis* administered (Drouault *et al*., 1999). These data confirm the protective nature of food matrices and demonstrate that a dairy-based delivery system in particular would favour lactococcal viability and survival in the GIT environment. *In vitro* analyses also suggest that the production of lacticin 3147 is not detrimental to the producing strain under simulated GIT conditions as there was no difference in the survival rates between *L. lactis* DPC6519 (bac$^-$) and *L. lactis* DPC6520 (bac$^+$).

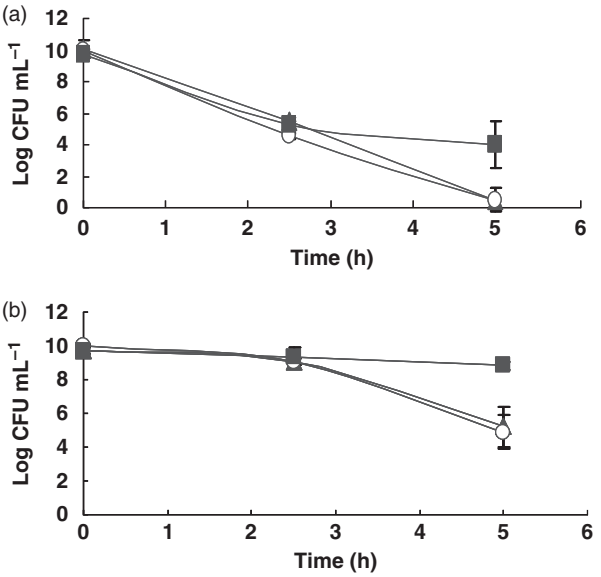

Fig. 2. Survival of *Lactococcus lactis* DPC6519 (▲), *L. lactis* DPC6520 (○) and *Lactobacillus salivarius* DPC6005 (■) in simulated ileum juice over time when suspended in MRD (a) or 10% RSM (b). Values are means of data from duplicate experiments, with SD indicated by vertical bars.

Survival of *L. lactis* DPC 6520 in the porcine GIT transit

The results outlined above demonstrate that when suspended in an appropriate matrix, lacticin 3147-producing lactococci are capable of surviving simulated gastrointestinal transit, albeit with some reduction in viability under small intestinal conditions. However, an accurate evaluation of the performance of lactococci in the gut can only be determined *in vivo*. To this end, a pig-feeding trial was conducted in order to evaluate the fate of lacticin 3147-producing lactococci in the mammalian GIT. The pig serves as a suitable model for humans due to the anatomical and physiological similarities of the GIT (Moughan *et al*., 1992). *Lactococcus lactis* DPC6520 (bac$^+$) was used exclusively in this trial, as simulation studies found no differences in survival between lacticin 3147-producing and -nonproducing strains. One group of pigs received a daily dose of 10^{11} CFU day^{-1} of *L. lactis* DPC6520 suspended in 10% RSM for 5 days, with a control group fed sterile 10% RSM. Faecal samples were taken before and during *L. lactis* administration and plated on a lacticin 3147- streptomycin-containing medium to enumerate the administered strain. In addition, bacteriocin activity assays and molecular fingerprinting by PFGE were used to confirm the identity of *L. lactis* DPC6520 (Fig. 3). Before culture administration, *L. lactis* DPC6520 was not detected in the faeces of any of the animals, although background levels of streptomycin-resistant, lacticin 3147-resistant bacteria were detected in both treatment and control pigs; however, PFGE analyses confirmed that these were not the strain administered (data not shown). Lacticin 3147–streptomycin-resistant colonies recovered from control pigs at day 5 were also shown not to be *L. lactis* DPC6520 (Table 1). Following 5 days of *L. lactis* DPC6520 administration, lacticin 3147–streptomycin-resistant colonies recovered from all four pigs fed the strain were confirmed by specific PCR amplification of the ltnαβ genes, lacticin 3147 production and PFGE analysis to be the administered *L. lactis* (Table 1). For three of these pigs, all representative colonies examined were confirmed as *L. lactis* DPC6520, demonstrating that the *L. lactis* strain

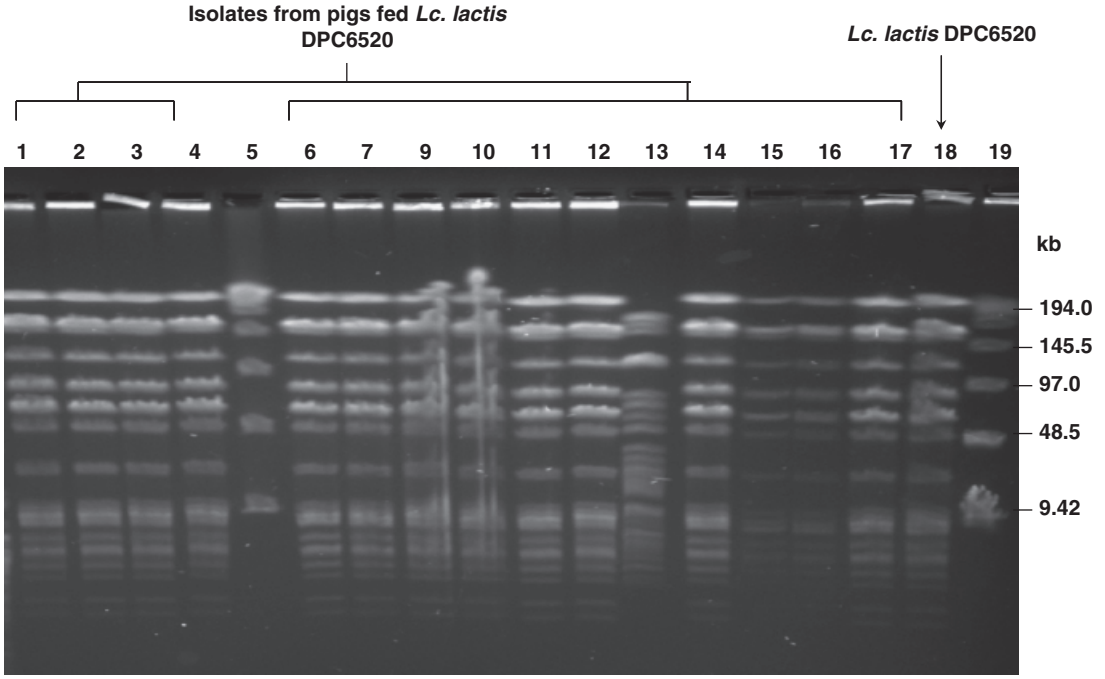

Fig. 3. PFGE patterns of representative faecal isolates obtained from selected pigs on day 5 of oral *Lactococcus lactis* DPC6520 administration (lanes 1–4 and 6–17) showing that all except the isolate in lane 13 are the administered strain, *L. lactis* DPC6520 (lane 18). Lanes 5 and 19 contain a 1000-kb λ ladder.

Table 1. Faecal counts of *Lactococcus lactis* DPC6520 in pigs following 5 days of administration of this strain at $\sim 10^{11}$ CFU day^{-1}

Pig no.	Group	Presumptive *L. lactis* DPC6520 counts (CFU g^{-1} faeces)*	No. of faecal isolates identified as producing lacticin 3147/total no. of isolates examined†	No. of faecal isolates identified by PFGE as administered strain/total no. of isolates examined
45	*L. lactis*	1.07×10^5	38/38	19/20
46	*L. lactis*	1.1×10^3	38/38	15/15
47	*L. lactis*	8×10^2	38/38	19/19
48	*L. lactis*	6.1×10^2	5/20	2/20
31	Control	ND‡	0/0	0/0
35	Control	8×10^2	0/4	0/0
37	Control	ND	0/0	0/0
38	Control	ND	0/0	0/0

Background counts from control pigs not fed *Lactococcus lactis* are also given. The proportion of day 5 faecal isolates identified by lacticin 3147 production and PFGE is shown.

*Counts were obtained on lacticin 3147–streptomycin-containing medium and are based on colony morphology.

†Activity against *L. lactis* ssp. *cremoris* HP, but not *L. lactis* ssp. *cremoris* HP (pMRC01), as determined in well diffusion assays.

‡ND, nondetectable. The limit of detection was 1×10^2 CFU g^{-1} faeces.

administered had survived gastrointestinal transit; although the counts varied from animal to animal (Table 1). However, in the fourth pig (no. 48), only 10% of the streptomycin–-lacticin 3147-resistant colonies tested proved to be the administered strain; consequently, no definitive *L. lactis* DPC6520 count could be obtained. These data highlight the variations in host–microorganism interactions and possibly microorganism–microorganism interactions that can govern gut microbiota proliferation (Zoetendal *et al.*, 2001). Furthermore, the excretion rates were relatively low when compared with the porcine probiotic *L. salivarius* DPC6005, which was recovered at $\sim 10^6$ CFU g^{-1} faeces when administered to pigs at 10-fold lower counts (Gardiner *et al.*, 2004).

Regardless of the fact that *L. lactis* DPC6520 survived intestinal transit, lacticin 3147 could not be detected in the faeces of any pig on day 5 using agar well diffusion assays. These results are in agreement with previous findings using

L. lactis CHCH2862, a nisin producer (Bernbom *et al.*, 2006), and *Lactobacillus plantarum* DEN 11007, a pediocin PA-1 producer (Bernbom *et al.*, 2009). In both of these studies, bacteriocin activity was not detected in the faecal samples of HMA rats fed the producing strains. Bacteriocin activity was only detected in the faeces when nisin or pediocin was administered as a concentrated supernatant or a purified preparation, most likely due to the high concentration of each bacteriocin administered. Indeed, upon finally reaching the colon, bacteriocin activity declined to just 2% of the initial pediocin PA-1 concentration administered and to 0.6% of the original nisin concentration. Moreover, when reconstituted lacticin 3147 skim milk was administered to pigs in a previous study, no lacticin 3147 activity was detected in faecal samples (Gardiner *et al.*, 2007). Therefore, if lacticin 3147 is produced by *L. lactis* DPC6520 within the gut, concentrations in the faeces may be quite low, considering the relatively small number of viable *L. lactis* cells that reach the colon and/or may be quickly degraded. As a result, bacteriocin concentrations may be below the limit of detection of most conventional bioassays such as the agar well diffusion assays used in this study. However, the use of specific procedures to extract lacticin 3147 may have facilitated bacteriocin recovery from faecal samples and should be considered in future experiments.

Survival of *L. lactis* in a human distal colon model and its effect on *C. difficile*

The trials in pigs demonstrated that lacticin 3147-producing lactococci are capable of surviving mammalian GIT transit, although lacticin 3147 was not detected in the faeces of any animal. Previous studies have demonstrated that *C. difficile* is extremely sensitive to lacticin 3147 (Rea *et al.*, 2007). Therefore, it is possible that even low concentrations of lacticin 3147, potentially produced by *L. lactis* in the GIT, may still exert an antimicrobial effect against this noninvasive, clinically relevant intestinal pathogen. Hence, the efficacy of *L. lactis* DPC6520 against *C. difficile* was investigated *ex vivo* in a simulated human distal colon model using *L. lactis* DPC6519 as a bacteriocin negative (bac^-) control. Viable counts of *L. lactis* DPC6520 (bac^+) decreased markedly during the 24-h faecal fermentation period, with a 10 000-fold reduction in cell numbers observed (from 2.2×10^9 to 6.6×10^4 CFU mL^{-1}; Fig. 4a). Viable counts of *L. lactis* DPC6519 (bac^-) also decreased (from 4.6×10^9 to 2.2×10^5 CFU mL^{-1}). It is likely that a combination of nutrient availability, temperature (37 vs. 30 °C) and competitive exclusion properties of the indigenous faecal microbiota contributed to this reduction in lactococcal viability. Moreover, no reduction in the mean *C. difficile* counts was seen throughout the experiment ($P > 0.05$; Fig. 4b). Although the counts increased slightly over the first 6 h in all vessels and decreased ~10-fold thereafter in the *L. lactis*-containing vessels only, the mean *C. difficile* counts over the 24-h fermentation period were not significantly different between the control vessel (1.1×10^7 CFU mL^{-1}) and the vessels containing *L. lactis* DPC 6520 (7.5×10^6 CFU mL^{-1}) or DPC 6519 (5.4×10^6 CFU mL^{-1}).

These data indicate that lactococci can survive in a human distal colon environment, but fail to proliferate, with reductions in viability observed within 24 h of administration. Whereas this phenomenon could be advantageous, releasing lacticin 3147 peptides into the colonic surroundings through cell lysis, this does not seem to be the case, as no significant inhibition of *C. difficile* was observed ($P > 0.05$). While we can only speculate as to the actual concentrations of lacticin 3147 produced (or not) within the colon model, no lacticin 3147 activity was detected in faecal slurry samples from the *L. lactis* DPC6520-containing vessel by the agar well diffusion assay. Furthermore, neither of the lacticin 3147 peptides was detected using MALDI–TOF MS analysis compared with a spiked control (Fig. 4c). This indicates that if lacticin 3147 is being produced, it is either rapidly adsorbed onto the surface of faecal bacteria or is in some way rendered inactive, possibly through enzymatic degradation. Recently, Kheadr *et al.* (2010) found that although pediocin PA-1 activity in a skim milk culture of *Pediococcus acidilactici* was detected following gastric digestion in an *in vitro* model of the GIT, no activity was detected in aliquots taken from simulated small intestinal compartments, indicating that pediocin is likely degraded within these environments. These results are also in agreement with previous findings that lacticin 3147 skim milk powder mixed with porcine faeces showed a marked reduction in activity as little as 1 h after initial exposure (Gardiner *et al.*, 2007).

Survival of *L. lactis* in the murine gut and its efficacy against *L. monocytogenes* infection

A mouse study was then performed in order to evaluate the effects, if any, of lacticin 3147-producing lactococci on the invasive intestinal pathogen *L. monocytogenes* in an established mouse model. Mice were fed a daily dose of 10^9 CFU of the lacticin 3147-producing strain, *L. lactis* DPC6520, or the nonproducing strain, *L. lactis* DPC6519, or no *L. lactis* (control) for a total of 5 days. On the third day, mice were challenged with 10^7 CFU of *L. monocytogenes* by oral gavage. To monitor excretion of the administered strains, daily faecal samples were taken and plated on lacticin 3147-streptomycin-containing medium to enumerate *L. lactis* DPC6520 (bac^+) and streptomycin-containing medium to enumerate *L. lactis* DPC6519 (bac^-). RAPD PCR was performed on selected colonies from faecal and caecal

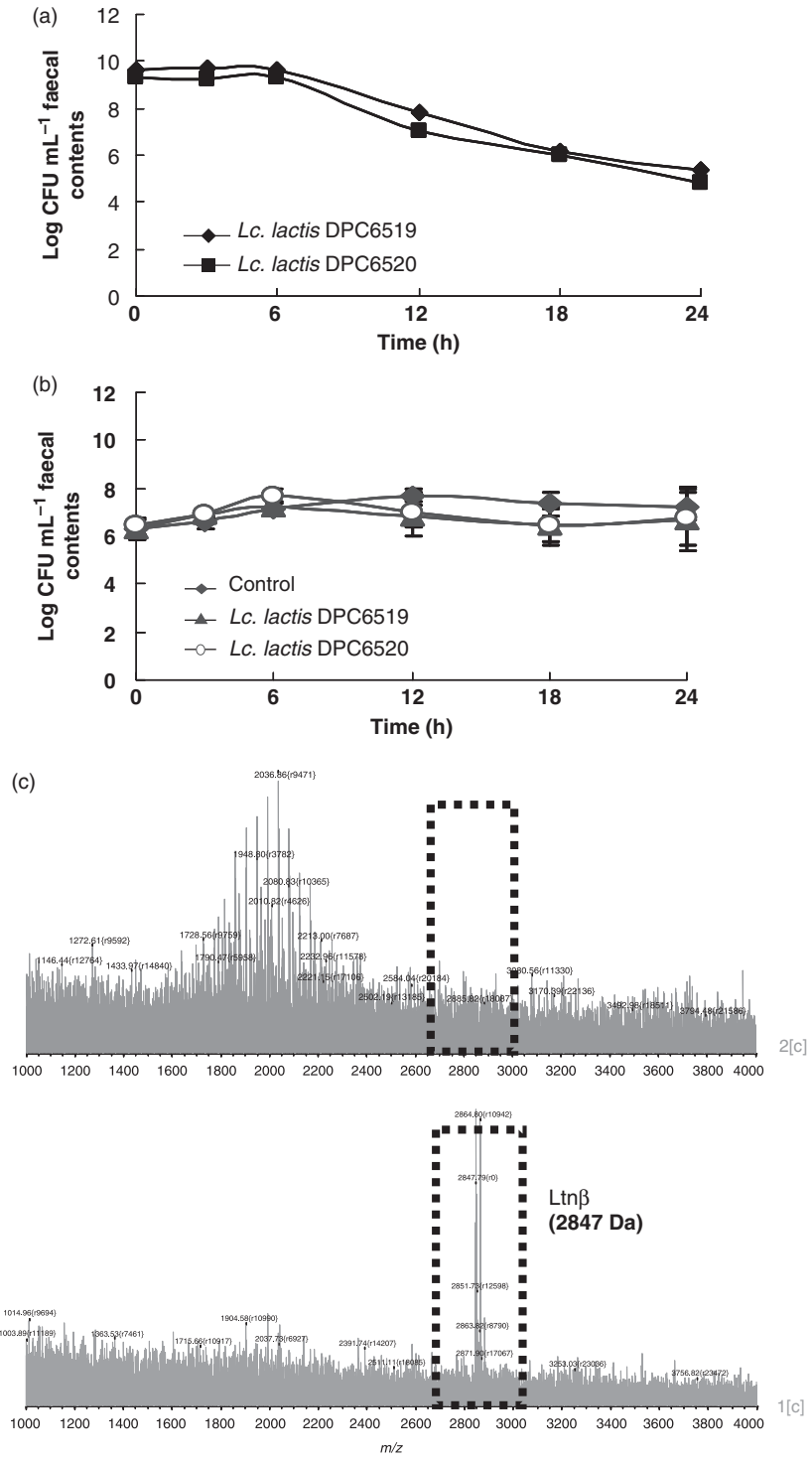

Fig. 4. (a) Survival of *Lactococcus lactis* DPC6520 (■) and *L. lactis* DPC 6519 (◆) in the simulated human distal colon model. (b) Survival of *Clostridium difficile* in the simulated human distal colon model upon the addition of 10^9 CFU of *L. lactis* DPC6519 (▲) or *L. lactis* DPC6520 (○) compared with the control vessel containing no *L. lactis* (◆). Values are means of data from duplicate experiments, with SD indicated by vertical bars. (c) MALDI–TOF MS analysis of an aliquot taken from an *L. lactis* DPC6520-containing vessel (top panel) compared with a control spiked with purified lacticin 3147 (bottom panel).

samples and confirmed the recovery of the administered strains. Lacticin 3147–streptomycin-resistant lactococci were not detected in any control mouse at any time-point. Over the 5-day administration period, the mean faecal excretion rates were significantly higher ($P < 0.05$) in *L. lactis* DPC6519-fed mice compared with mice administered *L. lactis* DPC6520 (7.1×10^5 vs. 4.7×10^4 CFU g^{-1} faeces; Fig. 5). Similarly, the mean caecal counts of *L. lactis* DPC6519 tended to be higher than *L. lactis* DPC6520 ($P = 0.06$), with DPC6519 recovered at 1.4×10^6 CFU g^{-1}

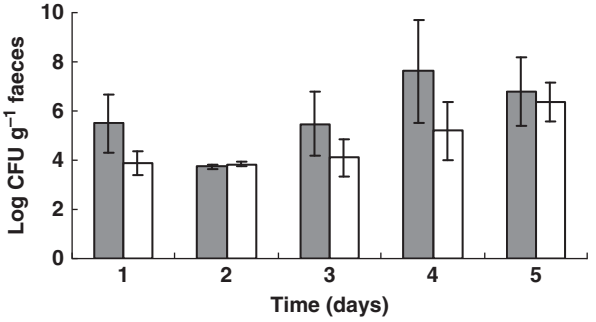

Fig. 5. Excretion of administered strains in mice fed 10^9 CFU day^{-1} *Lactococcus lactis* DPC6519 (solid bars) or *L. lactis* DPC6520 (open bars) in PBS over a period of 5 days. Values are the means of data from three to five mice, with SD indicated by vertical bars.

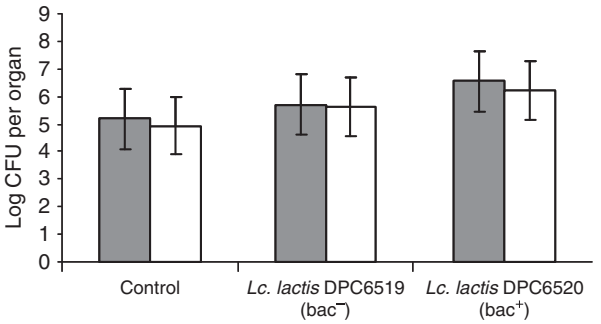

Fig. 6. Survival of *Listeria monocytogenes* EGDe in the liver (solid bars) and spleen (open bars) of mice administered *Lactococcus lactis* DPC6519 (bac$^-$), *L. lactis* DPC6520 (bac$^+$) or PBS for 3 days before *Listeria* infection. Values are the mean of data from five mice, with SE indicated by vertical bars.

caecal contents compared with 9.6×10^4 CFU g^{-1} for *L. lactis* DPC6520. However, there was no reduction in the *L. monocytogenes* counts in the spleen or the liver in mice fed either lacticin 3147-producing or -nonproducing lactococci compared with the control group ($P > 0.05$; Fig. 6). These results indicate that, although *L. lactis* is capable of surviving transit through the murine GIT, lacticin 3147-producing lactoccoci fail to confer any protection against *L. monocytogenes* infection. This is contrary to the findings of a study conducted by Millette *et al.* (2008) in which daily administration of a nisin-producing *L. lactis* strain for 16 days reduced intestinal vancomycin-resistant enterococci (VRE) colonization in mice. However, a control strain defective in nisin production was not used and the nisin-producing strain was not tracked *in vivo*; consequently, there was no conclusive evidence to show that the reduction in VRE counts could be solely attributed to bacteriocin production.

More convincing evidence of the efficacy of bacteriocin-producing strains *in vivo* has been demonstrated using the bacteriocin-producing strain *L. salivarius* UCC118 (Corr *et al.*, 2007). This study showed, via the use of a bacteriocin-negative mutant, that bacteriocin production was the primary mediator of protection against *Listeria* infection in mice. However, this is possibly due to the fact that *Lactobacillus*, a genus known to survive intestinal transit, was used for bacteriocin delivery. In agreement with our findings, a recent study has shown that the effect of pediocin-producing *P. acidilactici* on *Listeria* propagation in the murine intestine, liver and spleen was negligible (Dabour *et al.*, 2009). Although only a single dose of culture was administered, the authors attribute the lack of efficacy to the poor intestinal survival of the bacteriocin-producing strain, a factor that may also be responsible for the lack of anti-infective activity observed in our study. This hypothesis is further substantiated by suggestions that high lactococcal cell mortality rates in the small intestine reduce the potential of lactococci to produce significant amounts of therapeutic molecules within the GIT (Drouault *et al.*, 1999). Furthermore, the fact that ingestion of purified pediocin was able to confer protection against *Listeria* infection in the Dabour and colleagues study suggests that the bacteriocin-producing strain was either incapable of producing pediocin *in vivo* in an environment as complex as the GIT or could not produce it in adequate amounts to provide protection. This is also a likely explanation for the lack of *in vivo* efficacy in our study; indeed, a recent study from our laboratory has shown that purified lacticin 3147 effectively kills *C. difficile* in a manner similar to conventional antibiotics in a model of the distal colon (Rea *et al.*, 2011).

An additional pig-feeding trial showed that oral administration of *L. lactis* DPC6520 had no effect on faecal *Enterococcus* or *Lactobacillus* counts (data not shown). This provides further evidence for the lack of efficacy of lacticin 3147-producing *L. lactis* in the GIT, as we know that lacticin 3147 has drastic effects on the Gram-positive microbiota if present at sufficient concentrations (Rea *et al.*, 2007). However, because only a small proportion of the gut microbiota is cultivable (Zoetendal *et al.*, 2004), disturbances may still have occurred without being detected. To this end, a massive parallel sequencing strategy was used; samples were taken at 24 h from each of the simulated human distal colon experiments and total DNA was extracted. In this way, a total of 64 516 unique V4 variable regions of the 16S rRNA gene were amplified and sequenced. High-throughput sequencing revealed that of the assigned sequence reads in the control vessel, *Firmicutes* was the dominant phylum, accounting for 85% of the sequence tags in the control, followed by *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* (Fig. 7). These data are in agreement with previous studies using culture-independent approaches to study the microbial composition of the human GIT (Eckburg *et al.*, 2005; Tap *et al.*, 2009). Upon the addition of lacticin 3147-producing or -nonproducing lactococci to the

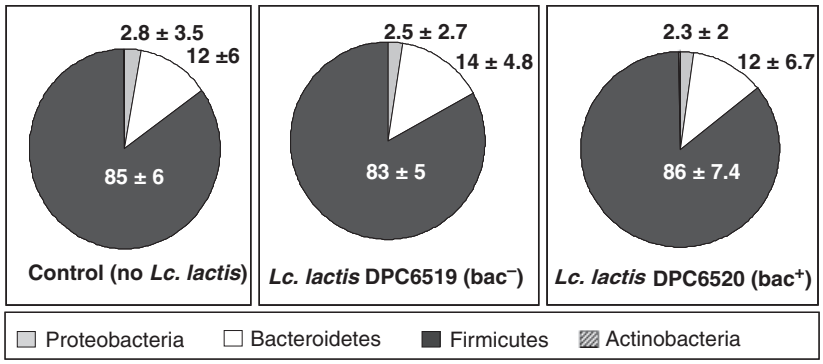

Fig. 7. Phylum-level taxonomic distribution of the microbial communities in a human distal colon model inoculated with *Clostridium difficile* upon the addition of *Lactococcus lactis* DPC6519 (bac$^-$) or *L. lactis* DPC6520 (bac$^+$) compared with the control vessel containing no *L. lactis*. Pie charts represent the mean distribution of sequences from two independent experiments and are expressed as the mean percentage of the total assignable tags recovered from each treatment vessel (± SD).

simulated distal colon model, no major disturbances in gut microbiota were observed at the phylum level. *Firmicutes* remained the dominant phylum, accounting for 83% and 86% of the total tag assignments in the vessels containing *L. lactis* DPC6519 (bac-) and *L. lactis* DPC6520 (bac+), respectively, while the overall percentages of both *Bacteriodetes* and *Proteobacteria* assignments remained relatively constant. It would be of interest to use these same parallel sequencing techniques to ascertain whether any intestinal microbiota changes occur as a result of the administration of lacticin 3147-producing *L. lactis in vivo*.

Conclusions

In-depth analysis of the antimicrobial efficacy of bacteriocin-producing microorganisms is of vital importance because little information currently exists about bacteriocin production and antagonism *in vivo*. In the present study, we have examined the efficacy of live *L. lactis* as a means of delivering functional bacteriocin peptides to the gut. We have demonstrated that bacteriocin-producing *L. lactis* are capable of surviving conditions that simulate the stomach, small intestine and colon, albeit with reductions in viability. Furthermore, intestinal transit survival was demonstrated *in vivo* both in mice and in pigs, although excretion rates were low and no lacticin 3147 activity was detected in the faeces. In addition, lacticin 3147-producing lactococci failed to attenuate *C. difficile* viability in an *ex vivo* model of the colon or to confer protection against *Listeria* infection in a mouse model. Taken together, these results show that lacticin 3147-producing *L. lactis* are capable of surviving mammalian GIT transit, albeit poorly, but that anti-infective activity is lacking *in vivo*. This may possibly be due to an inability of *L. lactis* to produce bacteriocin under intestinal conditions or production of insufficient amounts. Future work should perhaps focus on encapsulating the lacticin 3147-producing strain or developing more robust strains capable of proliferating under intestinal conditions as a means of delivering lacticin 3147 to the lower GIT *in vivo*.

Acknowledgements

The authors would like to thank Paula O'Connor as well as the staff of the Moorepark pig unit for technical assistance. This work was supported by the Science Foundation of Ireland funded Centre for Science, Engineering and Technology, the Alimentary Pharmabiotic Centre (APC).

References

Altschul SF, Madden TL, Schaffer AA *et al*. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**: 3389–3402.

Anderson DG & McKay LL (1983) Simple and rapid method for isolating large plasmid DNA from lactic streptococci. *Appl Environ Microb* **46**: 549–552.

Bernbom N, Licht TR, Brogren CH *et al*. (2006) Effects of *Lactococcus lactis* on composition of intestinal microbiota: role of nisin. *Appl Environ Microb* **72**: 239–244.

Bernbom N, Jelle B, Brogren CH, Vogensen FK, Norrung B & Licht TR (2009) Pediocin PA-1 and a pediocin producing *Lactobacillus plantarum* strain do not change the HMA rat microbiota. *Int J Food Microbiol* **130**: 251–257.

Berrada N, Lemeland JF, Laroche G, Thouvenot P & Piaia M (1991) Bifidobacterium from fermented milks: survival during gastric transit. *J Dairy Sci* **74**: 409–413.

Beumer RR, de Vries J & Rombouts FM (1992) *Campylobacter jejuni* non-culturable coccoid cells. *Int J Food Microbiol* **15**: 153–163.

Charalampopoulos D, Pandiella SS & Webb C (2003) Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. *Int J Food Microbiol* **82**: 133–141.

Conway PL, Gorbach SL & Goldin BR (1987) Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J Dairy Sci* **70**: 1–12.

Corr SC, Li Y, Riedel CU, O'Toole PW, Hill C & Gahan CG (2007) Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. *P Natl Acad Sci USA* **104**: 7617–7621.

Cotter PD, Hill C & Ross RP (2005) Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* **3**: 777–788.

Cotter PD, Deegan LH, Lawton EM *et al.* (2006) Complete alanine scanning of the two-component lantibiotic lacticin 3147: generating a blueprint for rational drug design. *Mol Microbiol* **62**: 735–747.

Dabour N, Zihler A, Kheadr E, Lacroix C & Fliss I (2009) *In vivo* study on the effectiveness of pediocin PA-1 and *Pediococcus acidilactici* UL5 at inhibiting *Listeria monocytogenes*. *Int J Food Microbiol* **133**: 225–233.

Drouault S, Corthier G, Ehrlich SD & Renault P (1999) Survival, physiology, and lysis of *Lactococcus lactis* in the digestive tract. *Appl Environ Microb* **65**: 4881–4886.

Eckburg PB, Bik EM, Bernstein CN *et al.* (2005) Diversity of the human intestinal microbial flora. *Science* **308**: 1635–1638.

Fallico V, McAuliffe O, Fitzgerald GF, Hill C & Ross RP (2009) The presence of pMRC01 promotes greater cell permeability and autolysis in lactococcal starter cultures. *Int J Food Microbiol* **133**: 217–224.

Fooks LJ & Gibson GR (2003) Mixed culture fermentation studies on the effects of synbiotics on the human intestinal pathogens *Campylobacter jejuni* and *Escherichia coli*. *Anaerobe* **9**: 231–242.

Ganzle MG, Hertel C, van der Vossen JM & Hammes WP (1999) Effect of bacteriocin-producing lactobacilli on the survival of *Escherichia coli* and *Listeria* in a dynamic model of the stomach and the small intestine. *Int J Food Microbiol* **48**: 21–35.

Gardiner G, Stanton C, Lynch PB, Collins JK, Fitzgerald G & Ross RP (1999) Evaluation of cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract. *J Dairy Sci* **82**: 1379–1387.

Gardiner GE, Casey PG, Casey G *et al.* (2004) Relative ability of orally administered *Lactobacillus murinus* to predominate and persist in the porcine gastrointestinal tract. *Appl Environ Microb* **70**: 1895–1906.

Gardiner GE, Rea MC, O'Riordan B *et al.* (2007) Fate of the two-component lantibiotic lacticin 3147 in the gastrointestinal tract. *Appl Environ Microb* **73**: 7103–7109.

Huson D, Auch A, Qi J & Schuster S (2007) MEGAN analysis of metagenomic data. *Genome Res* **17**: 377–386.

Kheadr E, Zihler A, Dabour N, Lacroix C, Le Blay G & Fliss I (2010) Study of the physicochemical and biological stability of pediocin PA-1 in the upper gastrointestinal tract conditions using a dynamic *in vitro* model. *J Appl Microbiol* **109**: 54–64.

Kimoto H, Nomura M, Kobayashi M, Mizumachi K & Okamoto T (2003) Survival of lactococci during passage through mouse digestive tract. *Can J Microbiol* **49**: 707–711.

Klaenhammer T, Altermann E, Arigoni F *et al.* (2002) Discovering lactic acid bacteria by genomics. *Antonie Van Leeuwenhoek* **82**: 29–58.

Klaenhammer TR (1993) Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev* **12**: 39–85.

Klijn N, Weerkamp AH & de Vos WM (1995) Genetic marking of *Lactococcus lactis* shows its survival in the human gastrointestinal tract. *Appl Environ Microb* **61**: 2771–2774.

Klostermann K, Crispie F, Flynn J, Meaney WJ, Paul Ross R & Hill C (2010) Efficacy of a teat dip containing the bacteriocin lacticin 3147 to eliminate Gram-positive pathogens associated with bovine mastitis. *J Dairy Res* **77**: 231–238.

Martin NI, Sprules T, Carpenter MR *et al.* (2004) Structural characterization of lacticin 3147, a two-peptide lantibiotic with synergistic activity. *Biochemistry* **43**: 3049–3056.

Millette M, Cornut G, Dupont C, Shareck F, Archambault D & Lacroix M (2008) Capacity of human nisin- and pediocin-producing lactic acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci. *Appl Environ Microb* **74**: 1997–2003.

Mills S, Coffey A, O'Sullivan L *et al.* (2002) Use of lacticin 481 to facilitate delivery of the bacteriophage resistance plasmid, pCBG104 to cheese starters. *J Appl Microbiol* **92**: 238–246.

Morgan SM, O'Connor PM, Cotter PD, Ross RP & Hill C (2005) Sequential actions of the two component peptides of the lantibiotic lacticin 3147 explain its antimicrobial activity at nanomolar concentrations. *Antimicrob Agents Ch* **49**: 2606–2611.

Moughan PJ, Birtles MJ, Cranwell PD, Smith WC & Pedraza M (1992) The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. *World Rev Nutr Diet* **67**: 40–113.

O'Connor EB, O'Riordan B, Morgan SM *et al.* (2006) A lacticin 3147 enriched food ingredient reduces *Streptococcus mutans* isolated from the human oral cavity in saliva. *J Appl Microbiol* **100**: 1251–1260.

O'Sullivan L, O'Connor EB, Ross RP & Hill C (2006) Evaluation of live-culture-producing lacticin 3147 as a treatment for the control of *Listeria monocytogenes* on the surface of smear-ripened cheese. *J Appl Microbiol* **100**: 135–143.

Pineiro M & Stanton C (2007) Probiotic bacteria: legislative framework – requirements to evidence basis. *J Nutr* **137**: 850S–853S.

Rea MC, Clayton E, O'Connor PM *et al.* (2007) Antimicrobial activity of lacticin 3147 against clinical *Clostridium difficile* strains. *J Med Microbiol* **56**: 940–946.

Rea MC, Dobson A, O'Sullivan O *et al.* (2011) Effect of broad and narrow spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. *P Natl Acad Sci USA* in press.

Ryan MP, Rea MC, Hill C & Ross RP (1996) An application in cheddar cheese manufacture for a strain of *Lactococcus lactis* producing a novel broad-spectrum bacteriocin, lacticin 3147. *Appl Environ Microb* **62**: 612–619.

Scannell AG, Ross RP, Hill C & Arendt EK (2000) An effective lacticin biopreservative in fresh pork sausage. *J Food Protect* **63**: 370–375.

Schloss PD & Handelsman J (2008) A statistical toolbox for metagenomics: assessing functional diversity in microbial communities. *BMC Bioinformatics* **9**: 34.

Simpson PJ, Stanton C, Fitzgerald GF & Ross RP (2002) Genomic diversity within the genus *Pediococcus* as revealed by randomly

amplified polymorphic DNA PCR and pulsed-field gel electrophoresis. *Appl Environ Microb* **68**: 765–771.

Stern NJ, Eruslanov BN, Pokhilenko VD *et al*. (2008) Bacteriocins reduce *Campylobacter jejuni* colonization while bacteria producing bacteriocins are ineffective. *Microb Ecol Health D* **20**: 74–79.

Tap J, Mondot S, Levenez F *et al*. (2009) Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol* **11**: 2574–2584.

Urich T, Lanzen A, Qi J, Huson DH, Schleper C & Schuster SC (2008) Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PLoS One* **3**: e2527.

Walsh MC, Gardiner GE, Hart OM *et al*. (2008) Predominance of a bacteriocin-producing *Lactobacillus salivarius* component of a five-strain probiotic in the porcine ileum and effects on host immune phenotype. *FEMS Microbiol Ecol* **64**: 317–327.

Zoetendal EG, Akkermans ADL, Akkermans-van Vliet WM, Arjan J, de Visser GM & de Vos W (2001) The host genotype affects the bacterial community in the human gastrointestinal tract. *Microb Ecol Health D* **13**: 129–134.

Zoetendal EG, Collier CT, Koike S, Mackie RI & Gaskins HR (2004) Molecular ecological analysis of the gastrointestinal microbiota: a review. *J Nutr* **134**: 465–472.