Title | Efficacy of a teat dip containing the bacteriocin lacticin 3147 to eliminate Gram-positive pathogens associated with bovine mastitis
---|---
Author(s) | Klostermann, Katja; Crispie, Fiona; Flynn, James; Meaney, William; Ross, R. Paul; Hill, Colin
Publication date | 2010
Type of publication | Article (peer-reviewed)
Link to publisher's version | http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=7496876&fulltextType=RA&fileId=S0022029909990239  
http://dx.doi.org/10.1017/S0022029909990239  
Access to the full text of the published version may require a subscription.
Rights | © Proprietors of Journal of Dairy Research 2009
Item downloaded from | http://hdl.handle.net/10468/1100
Downloaded on 2019-12-24T17:35:06Z
Efficacy of a teat dip containing the bacteriocin lacticin 3147 to eliminate Gram-positive pathogens associated with bovine mastitis

Katja Klostermann¹,², Fiona Crispie¹, Jim Flynn³, William J Meaney³, R Paul Ross¹,²* and Colin Hill²

¹ Moorepark Food Research Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland
² Department of Microbiology, University College Cork, Cork, Ireland
³ Dairy Production Research Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

Received 6 March 2009; accepted for publication 29 July 2009; first published online 29 September 2009

On most dairy farms teat dips are applied to the teats of cows either before or after milking in order to prevent pathogens from gaining access to the mammary gland via the teat canal. In the present experiments, a natural teat dip was developed using a fermentate containing the live bacterium Lactococcus lactis DPC 3251. This bacterium produces lacticin 3147, a two-component lantibiotic which was previously shown to effectively kill Gram-positive mastitis pathogens. Lacticin 3147 activity in the fermentate was retained at 53% of its original level following storage for 3 weeks at 4°C. In the initial experiments in vitro, 10⁵ colony-forming units/ml (cfu/ml) of either Staphylococcus aureus, Streptococcus dysgalactiae or Streptococcus uberis were introduced into the lacticin-containing fermentate. Neither Staph. aureus nor Str. dysgalactiae could be detected after 30 min or 15 min, respectively, while Str. uberis was reduced approximately 100-fold after 15 min. Following these trials, preliminary experiments were performed in vivo on teats of lactating dairy cows. In these experiments, teats were coated with each of the challenge organisms and then dipped with the lacticin-containing fermented teat dip. Following a dip contact time of 10 min, staphylococci were reduced by 80% when compared with the undipped control teat. Streptococcal challenges were reduced by 97% for Str. dysgalactiae and by 90% for Str. uberis. These trials showed that the teat dip is able to reduce mastitis pathogens on the teats of lactating cows.

Keywords: Mastitis prevention, teat dip, non-chemical, natural.

Mastitis is the most prevalent and costly disease in dairy herds, where it is associated with high somatic cell count (SCC) and decreased milk production. While many studies have tried to calculate economic losses caused by mastitis, the conclusions vary widely, as reviewed by Halasa et al. (2007). A study using national US data from 1996 found that the decrease in milk production associated with high bulk milk SCC caused a total loss of up to $810±480 million to the US economy when consumer supply and demand were taken into account (Losinger, 2005). In the UK, the annual value of output losses or resource wastage due to mastitis was estimated to have a direct cost of £121 million (Bennett et al. 1999). The same study also estimated annual treatment costs at an average of £61.5 million.

With respect to teat disinfection, teat dipping plays a crucial role in protection against invading mastitis pathogens and consists of treating teats pre- and/or post milking. It has been estimated that the implementation of teat dipping costs £1.40 per cow per year. The same study calculated that herds using three different preventive measures for mastitis, including teat dipping, could save an average of £35 per cow per year on disease costs, which would be a yearly saving of £3500 in a cow herd averaging 100 animals (Yalcin et al. 1999). Teat dips commonly contain diluted disinfectants, teat-skin conditioners or emollients as well as surfactants (Pankey et al. 1984). The antibacterial active ingredients used in teat dips are diluted disinfectants such as iodine or chlorhexidine. Use of a post-milking teat dip containing 1% iodine led to residues of 336 µg of iodine/l of milk marking a significant rise when compared with the corresponding value of 292 µg/l resulting from using 0.1% iodine in dips (Galton...
shown that preparations of the live producing culture against both these organisms (Ryan et al. 1999; Twomey et al. 2007) into teat seals improved the protection of animals from mastitis. In previous trials, we showed that incorporation of lacticin 3147 into teat dips made from a milk-based fermentate to eliminate the mastitis pathogens *Str. uberis*, *Str. dysgalactiae* and *Staph. aureus* both in vitro and in vivo on the teat surface. *Staph. aureus* and *Str. dysgalactiae* are predominant udder specific pathogens especially in Northern European countries (Barrett et al. 2005; Persson Waller et al. 2009) and the incidence rates of mastitis caused by such specific pathogens can be significantly reduced by the use of teat dipping (Whist et al. 2007). *Str. uberis* belongs to the group of environmental pathogens and is another important Gram-positive mastitis pathogen which can enter the teat canal after milking when the closure of the teat orifice is not yet established and the teat end is contaminated. The aim of the present study was to examine the bactericidal activity of a lacticin-containing fermentate against three important mastitis pathogens as a basis for the development of an effective teat dip in the future.

**Materials and Methods**

**Bacterial strains**

All bacterial strains were either selected from the culture collection at the Moorepark Food Research Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland or from the Bacteria Collection of the Laboratorium voor Microbiologie, Universiteit Gent, Belgium (Table 1). The lactococcal strains used in the study were *Lc. lactis* DPC 3147, the original strain found to produce the bacteriocin lacticin 3147 and *Lc. lactis* DPC 3251, another isolate which naturally overproduces the bacteriocin. *Lc. lactis* DPC 5399, a derivative of *Lc. lactis* DPC 3147 which does not contain the bacteriocin 3147-encoding plasmid pMRC01, was used as a lacticin negative control (*Bac*). *Lc. lactis* subsp. cremoris *HP* was used as a lacticin 3147-sensitive indicator strain. All lactococcal strains were maintained on M17 Agar or Broth (Becton Dickinson and Company, Sparks MD, USA) supplemented with 0.5% (w/v) Lactose (LM17 Agar and LM17 Broth). The lactococcal strains were grown overnight at 30°C.

The mastitis pathogen *Staph. aureus* DPC 5246 was isolated from a naturally occurring mastitis case and was used in previous studies as a challenge organism (Twomey et al. 2000; Crispie et al. 2005). *Str. uberis* Diernhofer 1932 and *Str. dysgalactiae* M were also isolates from mastitic milk and the latter was used as a challenge strain in experiments both in vivo and in vitro (Ryan et al. 1999). All mastitis pathogens were routinely grown in TSB (Tryptic Soy Broth, B.D. and Co., 38800 Le Pont de Claix, France) and on Blood Agar Base No. 2 (Merck, Darmstadt, Germany) supplemented with 0.1% aesculin (w/v, Sigma Chemical Company, St. Louis MO, USA) and 5–7% (v/v) whole calf blood (Aesculin Blood Agar, ABA) (IDF, 1981).
Staphylococci were enumerated on Mannitol Salt Agar (Merck, Darmstadt, Germany) while streptococci were grown on Modified Edwards Medium (Oxoid Ltd., Hampshire, England) supplemented with 5–7% (v/v) whole calf blood (IDF, 1981).

### Preparation of the teat dip

The teat dip was prepared by growing *Lc. lactis* DPC 3251 in 10% (w/v) reconstituted skim milk (10% RSM). An overnight broth culture of the above strain was used to inoculate 10% RSM and was subsequently grown for 20 h at 30°C. The resulting fermentate was then stored at 4°C and used as the 3251 teat dip in bactericidal assays and in experiments in vivo. For the experiments in vitro, a control fermentate produced by the bacteriocin negative strain *Lc. lactis* DPC 5399 was prepared using identical conditions to the 3251 fermentate. The fermentate of the strain *Lc. lactis* DPC 5399 (Bac– fermentate) was chosen because it is a bacteriocin-negative derivative of the original bacteriocin producer *Lc. lactis* DPC 3147. The pH and the consistency of the 5399 fermentate were similar to fermentsates produced by the lacticin-overproducing strain *Lc. lactis* DPC 3251. Both dips were always used within 24 h of production.

### Lacticin production and storage stability

To assess the storage stability of the bacteriocin in the *Lc. lactis* 3251-fermentate at 4°C, the lacticin activity of a 3251 fermentate, prepared in duplicate, was assayed over a 3-week period on days 1, 4, 7, 14 and 21. Bacteriocin activity was measured by an agar well diffusion assay, with the lacticin 3147-sensitive strain *Lc. lactis* subspecies *cremoris* HP (Table 1) as the indicator strain. Activity was measured and expressed as arbitrary units (AU/ml) as described by Ryan et al. (1996).

### Preparation of challenge bacteria for bactericidal assays and teat challenges

The challenge bacteria were prepared by inoculating overnight cultures of *Staph. aureus* DPC 5246, *Str. uberis* Diernhofer 1932 or *Str. dysgalactiae* M to 1% (v/v) in TSB and incubating at 37°C for 16 h. Resulting cultures were then diluted to a concentration of 10^5 cfu/ml using TSB and these dilutions were immediately used as the challenge in bactericidal assays. Initially, challenge concentrations of 10^5 cfu/ml were also used for experiments in vivo. To ensure sufficient recoveries to allow accurate assessment of effectiveness for experiments in vivo, the concentration of challenge organisms had to be increased to >10^7 cfu/ml.

### Bactericidal assays measuring the effect of teat dips on Gram-positive mastitis pathogens

The teat dip was assessed for its ability to kill Gram-positive mastitis pathogens in vitro. The Bac– fermentate served as the control dip. In each assay, 1 ml of the diluted challenge pathogen was mixed with 10 ml of the teat dip. Immediately after the addition of the challenge to the dip solution, dips were mixed thoroughly. An aliquot of the challenge strain was taken immediately (time zero, T0) for viability counts and the mixture of teat dip and challenge pathogen was incubated at 37°C, similarly to experiments by Koskinen et al. (1996). Ryan et al. (1998) previously showed that lacticin 3147 was generally more potent against streptococci than staphylococci and thus, as staphylococcal challenges were expected to be more resilient than streptococci, aliquots in such challenges were taken after 30, 60, 120 and 180 min (T30, T60, T120 and T180). To examine expectedly quicker changes, aliquots for streptococcal challenges were taken after 15, 30, 60 and 120 min (T15, T30, T60 and T120). Each aliquot was immediately diluted and 100 μl of each dilution as well as 100 μl of the original aliquot were plated in duplicate using standard microbiological procedures as described by Twomey et al. (2000). Plates were incubated overnight at 37°C and viable challenge pathogens were counted. The detection of pathogen activity was very sensitive and pathogens ≥10 cfu/ml could be detected. If no growth was detected on any of the plates (numbers of pathogens <10 cfu/ml) then a value of zero was applied (Twomey et al. 2000), represented as 0 log_{10} cfu/ml (Fig. 3).

### Table 1 Summary of bacterial strains

<table>
<thead>
<tr>
<th>Strain Description Source or Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural overproducer of lacticin 3147</td>
</tr>
<tr>
<td>Lacticin 3147 negative strain</td>
</tr>
<tr>
<td>Indicator strain, sensitive to lacticin 3147</td>
</tr>
<tr>
<td>Bovine mastitis clinical isolate</td>
</tr>
<tr>
<td>Bovine, mastitis milk</td>
</tr>
<tr>
<td>Bovine mastitis clinical isolate</td>
</tr>
</tbody>
</table>

† Moorepark Food Research Centre
‡ Bacteria Collection of the Laboratorium voor Microbiologie, Universiteit Gent, Belgium
Selection of animals, sampling procedures and microbiological methods for trials in vivo

For the trials in vivo, live teats rather than excised teats were used to mimic field conditions, as the Bac⁺ fermentate, and the challenge pathogens may be affected by the changing temperatures of the teat surface after milking (Isaksson & Lind, 1994) or by other conditions found on live teats, which can be overlooked using an excised teat model. Eighteen teats of eight lactating Holstein-Friesian cows were used for the teat dip trials. Of these, five teats were assigned to the trial with the staphylococcal challenge. Seven teats and a further six teats were assigned to the trials with Str. dysgalactiae M and Str. uberis Diernhofer 1932, respectively. One teat in each of the three trials served as an undipped control. To identify teats suitable for the experiments, quarter milk samples of cows were collected aseptically from all trial cows before the morning (AM) milking. For the latter sample collection, teats were cleaned by washing with water and then dried with service-paper towels. Immediately before sampling, teats were then disinfected with cotton wool swabs containing Methylated Spirits (Blackhall Pharmaceutical Distributors Ltd., Swords, Co. Dublin, Ireland). Twenty-five ml of milk were collected from each quarter. SCC of milk samples was determined using the Somacount 300° (Bentley Instruments Incorporated, USA). Pathogens in milk samples were detected by plating 10⁻³ cells/ml and were free of pathogens.

Additional, the selected teats were free from warts, lesions, crusts or abrasions, showed a uniform size, with smooth teat skin and no signs of inflammation (redness, tenderness or swelling).

Challenge, teat dipping procedure and recovery of challenge bacteria used in trials in vivo

Following the AM milking, the selected teats and the surrounding skin were prepared for the trial by washing with lukewarm water. Teats and udder were subsequently dried with service paper towels. Washed teats were then disinfected twice from teat base to tip with cotton wool swabs containing Methylated Spirits® (Blackhall Pharmaceutical Distributors Ltd). The challenge and teat dipping procedures were essentially a modified version of the procedure described by Philpot et al. 1978. The principal dipping technique is shown in Fig. 1. In brief, the washed and disinfected teats (including control teats) were dipped once in 25-ml beakers containing 12-ml volumes of the challenge culture. The teat tip had to touch the bottom of the beaker to ensure equal coating of each teat with the challenge organism. Following a 5-min drainage time, each of the teats selected for dipping with the DPC

Fig. 1. Technique for challenge, teat dip, wash off and recovery on live teats

3251 teat dip was submerged into a beaker containing 20 ml of the experimental dip, while one teat per organism type remained undipped as a negative control. The contact time for the teat dip was 10 min. After the contact of the challenge organisms with the dip, the surviving organisms were recovered by submerging the teats (control and dipped teats) into sterile beakers containing 20 ml MRD (Maximum Recovery Diluent, Oxoid Ltd., Hampshire, England). The final recovery suspension from each teat was then stored in Sterilin® bottles and immediately transported to the laboratory to enumerate the surviving bacterial cells. The recovery solutions were diluted and plated in duplicate within 2 h of collection. Plates were incubated overnight at 37 °C and viable challenge pathogens were counted.

Aftercare of the animals

To remove any remaining bacteria from the teat surface, all teats were disinfected with cotton wool swabs soaked in Methylated Spirits® after the final recoveries were taken. The teats were then washed with lukewarm water to remove any teat-dip residues. Teats were subsequently dried with service paper towels. Finally, the teats were sprayed with a commercial teat dip, Deosan Summer Teatcare Plus® (RTU, Diversey Lever) which was used routinely in the herd.

Results

Preparation of the fermented teat dip and lacticin content throughout storage

Teat dips used in all the experiments consisted of the described fermentate of Lc. lactis DPC 3251 grown in 10% RSM (Bac⁺ fermentate). Generally, teat dips produced by growing Lc. lactis DPC 3251 contained bacterial counts
of $10^6$ cfu/ml after 20-h incubation at 30°C. The lacticin activity for each Bac+ fermentate used in bactericidal assays or live teat challenges was $\geq 10^{240}$ AU/ml at a pH value of approx. 4.4. No lacticin activity was detected in Bac− fermentates and the pH value of the control fermentates was always similar to that of the lacticin-containing fermentate at approx. 4.4.

The lacticin stability of two dips was assessed during storage of the dip at 4°C for up to 21 d. The mean lacticin activity in the dips declined to 60% in the first 7 d of storage (Fig. 2) but the bacteriocin still retained 53% of its activity until the final sampling day.

**Bactericidal assays against Gram-positive mastitis pathogens**

The anti-bacterial efficacy of the fermented teat dip was first evaluated ex vivo against *Staph. aureus* DPC 5246, *Str. dysgalactiae* M and *Str. uberis* Diernhofer 1932. A control fermentate produced by the Bac− strain *Lc. lactis* DPC 5399 was included in the assays. When exposed to the Bac+ fermentate, *Staph. aureus* DPC 5246 was rapidly killed and no live cells were recovered after 30 min of incubation (a reduction of 5.2 log10 cfu/ml). In contrast, the Bac− fermentate resulted in a decrease in viability of only 1.6 log10 cfu/ml (Fig. 3). The Bac+ fermentate therefore resulted in a reduction of staphylococci of 3.6 log10 cfu/ml compared with the Bac− fermentate. Similarly, when *Str. dysgalactiae* M was incubated with the Bac+ teat dip, no viable pathogens could be detected after 15 min (a reduction of at least 5 log10 cfu/ml; Fig. 3). Given that the Bac− fermentate resulted in a reduction of only 0.7 log10 cfu/ml we conclude that the majority of the bactericidal action of the dip is due to the lacticin content. In contrast, *Str. uberis* Diernhofer 1932 seemed to survive better in the Bac+ dip, where numbers were reduced by approximately 2 log10 cfu/ml in the first 15 min ($T_0$-$T_{15}$). After 120 min some live cells were still present albeit at a very low numbers (approx. 60 cfu/ml). Again, *Str. uberis* Diernhofer 1932 was only slightly reduced in the Bac− control solution (Fig. 3).

**Efficacy of the lacticin-based teat dip in vivo**

To evaluate the potential value of performing large scale animal trials, we examined the short-term effect of the Bac+ fermentate on live teats of lactating dairy cows. For this purpose three separate, small scale teat dip trials were performed in which five teats were challenged with staphylococci, seven with *Str. dysgalactiae* and a further six with *Str. uberis*. To gain sufficient bacterial recoveries for monitoring purposes, challenge concentrations for these trials were set greater than $10^7$ cfu/ml. When *Staph. aureus* DPC 5246 was used as the challenge organism, mean recoveries of viable staphylococci on the teat dipped with the DPC 3251 dip were 4.68 log10 cfu/ml. Compared with the recoveries from the untreated control teat, this represented an 80±8% elimination of the challenge organism on the teat surface (Fig. 4). The mean streptococcal recovery after dipping with the DPC 3251 teat dip was 2.68 log10 cfu/ml for *Str. dysgalactiae* M and the mean elimination was found to be 97.5±2.5% (Fig. 4). Mean recoveries from teats challenged with *Str. uberis* Diernhofer 1932 were 3.8 log10 cfu/ml, representing an elimination of 90.2±5.3% (Fig. 4). No animal or quarter enrolled showed any signs of infection with the pathogens used for the challenge procedure after the trial.
both the resultant dip was shown to be highly effective in killing the specific udder pathogens that have been shown previously that the specific udder pathogens of which is associated with the energy state of the cell. Alternatively, it should be noted that as Staph. aureus growth rates of the environmental pathogen might suggest that a lacticin-based teat dip would be more effective on the teat surface than a similarly manufactured nisin-based dip, although this would need to be tested experimentally.

In a study by Oliver & Mitchell (1985), a natural preparation made from Lactobacillus acidophilus reduced numbers of staphylococci in skim milk at neutral pH only showing best effects against Staph. aureus when incorporated into a teat seal formulation, whereas lacticin was able to show a clear zone of inhibition (Ryan et al. 1998). Similarly, nisin was unable to reduce numbers of staphylococci in skim milk at neutral pH only showing best effects against Staph. aureus under low-pH conditions (Sobrino-Lopez & Martin-Belloso, 2006). These results might suggest that a lacticin-based teat dip would be more effective on the teat surface than a similarly manufactured nisin-based dip, although this would need to be tested experimentally.

In a study by Oliver & Mitchell (1985), a natural preparation made from Lactobacillus acidophilus reduced numbers of Staph. aureus and streptococcal species by 72% and >95%, respectively. These numbers are comparable to the efficacy of the 3251 fermentate in the present study, as staphylococci were reduced by approx. 80% and average streptococcal reductions were approx. 96%. Sears et al. (1992) found that purified nisin reduced Staph. aureus on the teat skin of live cows by 3-9 log_{10} CFU/ml after an exposure of 1 min compared with a reduction of 0-7 log_{10} CFU/ml for staphylococci in the present study after a contact time of 10 min. It should be emphasised, however, that a preparation based on purified or highly concentrated nisin may prove expensive for such applications. Additionally, it should be noted that as Staph. aureus strains can vary significantly in virulence potential (Dingwell et al. 2006) and indeed in susceptibility to lacticin 3147 itself (Ryan et al. 1998), our results cannot be compared directly with the results with nisin as different isolates of Staph. aureus were used.

Another factor which should be considered in the analysis of our results is the absence of any surfactants or emollients in the dips described in this study. Surfactants and emollients are used in commercial teat disinfectants in order to ensure proper coating of the teat surface and these two mastitis pathogens in milk can be higher than growth rates of the environmental pathogen Str. uberis (Oz et al. 1986). Thus, as lacticin activity depends on the energy status of the target cell, this may explain why Staph. aureus and Str. dysgalactiae are killed more effectively than the slow-growing Str. uberis in the milk-based environment of the fermented dip. A longer incubation period might have resulted in a more complete elimination of Str. uberis. However, as Staph. aureus is the most frequently isolated strain in Irish dairy cows with high SCC (Barrett et al. 2005) we want to emphasize that the effects of the teat dip resulted in a complete elimination of Staph. aureus.
to keep the teat skin smooth and moisturized. One such surfactant and one emollient have been integrated into the 3251 dip in other assays. Addition of the surfactant Texapon® (TEA Lauryl Sulphate, courtesy of Cross Vet, Research Laboratories, Ireland) at a concentration of 1% (v/v) or the emollient Glycerol® (Sigma Aldrich Ireland Ltd., Dublin, Ireland) at a concentration of 5% (v/v) to the lacticin teat dip did not increase the average antibacterial effectiveness in these assays when compared with results of the teat dip without additives (data not shown). However, more studies should be undertaken to determine whether other surfactants could enhance the activity of the lacticin-based dip.

It was previously found that under varying natural conditions streptococcal and staphylococcal counts on teats never exceeded approx. 3 or 4 log_{10} cfu/ml respectively (Hogan et al. 1990). Therefore, artificial teat challenge models present a much higher bacterial count to any experimental teat dip than in conditions found under natural exposure. Therefore under natural conditions, the dip may be even more effective. Another parameter which may have influenced the results of the activity of the dips was the incubation temperature of 37 °C used when recovering pathogens. Although the resting skin surface temperature of the teat in a housed cow averages 30 °C, it has been shown that during milking, the temperature of the teat surface can be subject to change and can range from 29 °C to 38 °C (Isaksson & Lind, 1994). The surface temperature is also higher after milking when the milk flow has warmed the teat surface (Isaksson & Lind, 1994). Therefore, as teat dips are applied immediately post milking, when the milk flow has warmed the teat surface, it was decided that similarly to Koskinen et al. (1996), for the bactericidal assays in vitro, an incubation temperature of 37 °C should be used. This may also have positively selected for pathogens as this temperature is recommended by National Mastitis Council for optimal recovery of pathogens and is also substantially higher than the optimum growth temperature for *Lc. lactis* DPC 3251 of 30 °C (S Morgan, personal communication). To examine the 3251 dip on a larger scale, a natural exposure trial, also evaluating the effectiveness of the dip at varying temperatures, would therefore be recommended. In the natural exposure trial by Koskinen et al. (1996) fewer isolates of *Staph. aureus* (2-09%) or coagulase-negative staphylococci (2-52%) were found in milk from udder quarters dipped with sour milk compared with the results of undipped controls (3-09% and 4-07% of total isolates, respectively). The group dipped with an iodine-containing dip had the least number of *Staph. aureus* isolates (0-83% total isolates); however, there were no statistical significant differences between treatments. Following their natural exposure trial, Oliver & Mitchell (1985) concluded that their *Lb. acidophilus* preparation was effective in preventing intramammary infections by 57% when compared with the number of infections in the undipped control group. Additionally, no teat irritations were noticed. In a field trial evaluation by Sérieys & Poutrel (1996) a commercial dip formulation containing nisin was compared with a 0-5% iodine dip. No significant difference in new infection rates was found. Another non-chemical teat dip based on an Aloe Vera gel was also compared with an iodine dip in a field trial. Results showed that the mastitis occurrence in both groups was not significantly different (Leon et al. 2004). These results have therefore shown that to date, alternative (*‘non-chemical’*) teat dips have proven efficient in preventing mastitis.

From a food perspective, consumers are becoming more aware of the sources, contents and the production of food, which is evident from the rising demands for organic and natural dairy products (McElroy, 2008). In this respect, a natural, non-chemical teat dip containing food grade bacteria would certainly be of benefit to the dairy industry. Additionally, given that chemical teat dips can cause severe irritation of the skin (Sears et al. 1992), the search for milder agents with antimicrobial action seems to be a sensible approach. In the present study, the teat skin effect of an application of the 3251 dip was not examined, though in the long term trials by Koskinen et al. (1996) and Oliver & Mitchell (1985) using similar products, no adverse effects on the teat skin with either teat dip were mentioned.

If teat dips containing food grade bacteria were to be commercialized, problems with increases in total bacteria count (TBC) might arise as levels of, in this case, *Lc. lactis* DPC 3251 in the milk might lead to a rise in the resulting bacterial count. Dairy co-operatives, however, could use differential counting of TBC for dairy farms using such a teat dip. The effect of milk containing LAB on processing would also require investigation, though the process of pasteurization, which would eliminate any remaining lactococci, is used in most dairy processing plants and raw milks are rarely used.

In conclusion, the lacticin-containing 3251 teat dip used in this study decreased Gram-positive mastitis pathogens under laboratory conditions as well as on the teats of live cows. Further investigations in accordance with National Mastitis Council recommendations (Nickerson et al. 2004) would be required in order to produce a commercially viable product.

References
Arbesman H 2005 Dairy and acne-the iodine connection. *Journal of the American Academy of Dermatology* 53 1102
Crispie F, Twomey D, Flynn J, Hill C, Ross P & Meaney W 2005
The lantiibiotic lacticin 3147 produced in a milk-based medium improves
the efficacy of a bismuth-based teat seal in cattle deliberately
infected with Staphylococcus aureus. Journal of Dairy Research
72 159–167

Academy of Dermatology 56 164–165

genotype of Staphylococcus aureus, determined by pulsed-field gel
electrophoresis, on dry-period elimination of subclinical mastitis in
Canadian dairy herds. Canadian Journal of Veterinary Research 70
115–120

Fisher DA 1989 Upper limit of iodine in infant formulas. Journal of
Nutrition 119 1865–1868

Galton DM, Peterssson LG & Erb HN 1986 Milk iodine residues in herds
practicing iodophor premilking teat disinfection. Journal of Dairy
Science 69 267–271

Halasa T, Huijps K, Østera˚s O & Hogeveen H 2007 Economic effects of
bovine mastitis and mastitis management: a review. Veterinary
Quarterly 29 18–31

Hogan JS, Smith KL, Todhunter DA & Schoenberger PS 1990 Bacterial
counts associated with recycled newspaper bedding. Journal of Dairy
Science 73 1758–1761

International Dairy Federation 1981 Chapter 3: Isolation and identifi-
cation of mastitis bacteria. In: Laboratory methods for use in mastitis
work, Bulletin 132 19–26

Isaksson A & Lind O 1994 Brief Communication: Milking-related changes
in the surface temperature of the bovine teat skin. Acta Veterinaria
Scandinavica 35 435–438

Intramammary infusion of a live culture of Lactococcus lactis for
treatment of bovine mastitis: comparison with antibiotic treatment in
field trials. Journal of Dairy Research 75 365–373

Koskinen E, Rantala M & Saloniemi H 1996 The effect of sour milk as a
postmilking teat dip for mastitis prevention in a dairy herd. Acta
Veterinaria Scandinavica 37 427–432

Leon L, Beer C, Wacken H, Nurnberg M & Andersson R 2004 [Effect on
teat skin condition and efficacy against new intramammary infec-
tions of a barrier teat dip based on aloe vera gel.] Tierärztliche
Umschau 59 237–244

Losinger WC 2005 Economic impacts of reduced milk production asso-
ciated with an increase in bulk-tank somatic cell count on US
dairies. Journal of the American Veterinary Medical Association 226
1652–1658

Lacticin 3147, a broad-spectrum bacteriocin which selectively dis-
sipates the membrane potential. Applied and Environmental Micro-
biology 64 439–445

McElroy B 2008 Global statistics of the organic market. Ecological
Farming Conference, Pacific Grove CA, USA.

Nickerson SC, Saxon A, Fox UK, Hemling T, Hogan JS, Morelli J,
Oliver SP, Owens WE, Pawlak M & Petersson L 2004 National
Mastitis Council: Recommended protocols for evaluating efficacy of
postmilking teat germicides. In: NMC Annual Meeting Proceedings
pp. 379–399

Oliver SP & Mitchell BA 1985 Prevention of bovine mastitis by a
Lactobacillus acidophilus preparation. Journal of Dairy Science 68
271

Oz HH, Farnsworth RJ & Cox HU 1986 Growth of Gram-positive mast-
togenic bacteria in normal, simulated bulk tank, and mastitic milk held
at simulated fluctuating temperatures of farm bulk tank. Journal of
Dairy Science 69 2060–2065

Pankey JW, Eberhart RJ, Cuming AL, Daggett RD, Farnsworth RJ &
Science 67 1336–1353

Park YK, Harland BF, Vanderveen JE, Shank FR & Prosky L 1981
Estimation of dietary iodine intake of Americans in recent years.
Journal of the American Dietetic Association 79 17–24

Pennington JA, Young RE & Wilson DB 1989 Nutritional elements in
American Dietetic Association 89 659–664

Persson Waller K, Bengtsson B, Lindberg A, Nyman A & Ericsson H 2009
Incidence of mastitis and bacterial findings at clinical mastitis in
Swedish primiparous cows—influence of breed and stage of lactation.
Veterinary Microbiology 134 89–94

Philpot WN, Boddie RL & Pankey JW 1978 Hygiene in the prevention of
udder infections. IV. Evaluation of teat dips with excised cows’ teats.
Journal of Dairy Science 61 950–955

Irish Scientist 2 7

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, lactin 3147. Applied and Environ-
mental Microbiology 62 612–619

Ryan MP, Meaney WJ, Ross RP & Hill C 1998 Evaluation of lactin 3147
and a teat seal containing this bacteriocin for inhibition of mastitis
pathogens. Applied and Environmental Microbiology 64 2287–2290

Ryan MP, Flynn J, Hill C, Ross RP & Meaney WJ 1999 The natural food
grade inhibitor, lactin 3147, reduced the incidence of mastitis after
experimental challenge with Streptococcus dysgalactiae in nonlactat-
ing dairy cows. Journal of Dairy Science 82 2625–2631

Sears PM, Smith BS, Stewart WK, Gonzalez RN, Rubino SD, Gusik SA,
Kulisik ES, Projan SJ & Blackburn P 1992 Evaluation of a nisin-based
germicidal formulation on teat skin of live cows. Journal of Dairy
Science 75 3185–3190

Serfies F & Poutrel B 1996 Field trial evaluation of two teat dips con-
taining nisin or polyvinylpyrrolidone iodophor designed for use before
and after milking. Veterinary Research 27 295–303

Sit CS & Vederas JC 2008 Minireview: Approaches to the discovery of
new antibiotic agents based on bacteriocins. Biochemistry and Cell
Biology 86 116–123

Sobrino-Lopez A & Martín-Belloso O 2006 Enhancing inactivation of
Staphylococcus aureus in skim milk by combining high-intensity
pulsed electric fields and nisin. Journal of Food Protection 69 345–353

Protection against Staphylococcus aureus mastitis in dairy cows using
a bismuth-based teat seal containing the bacteriocin, lactin 3147.

Vanderveen JE 1979 Estimation of total iodine consumption by Americans
in recent years. Presented at: Iodine Workshop, American Medical
Association, Scottsdale, Arizona, November 13–14

Wiedemann I, Bottiger T, Bonelli RR, Wiese A, Hagge SO, Gutmann T,
Seydel U, Deegan L, Hill C, Ross P & Sahl HG 2006 The mode of
action of the lantiibiotic lactin 3147-a complex mechanism involving
specific interaction of two peptides and the cell wall precursor lipid II.
Molecular Microbiology 61 285–296

Whist AC, Østera˚s O & Solverod L 2006 Staphylococcus aureus and
Streptococcus dysgalactiae in Norwegian herds after introduction of
selective dry cow therapy and teat dipping. Journal of Dairy Research
74 1–8

Yalcin C, Stott AW, Logue DN & Gunn J 1999 The economic impact of
mastitis-control procedures used in Scottish dairy herds with high
bulk-tank somatic-cell counts. Preventative Veterinary Medicine 41
135–149