Production of antilisterial bacteriocins by staphylococci isolated from bovine milk¹

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ABSTRACT

A collection of 111 staphylococcal isolates recovered from healthy cows in 41 dairy herds in Brazil was surveyed for the production of bacteriocins. The group included 94 coagulase-positive and 17 coagulase-negative strains of staphylococci. All cultures were grown in tryptic soy broth for 18 h at 37°C, and cell-free supernatants were tested for antimicrobial activity against several target organisms by using the agar diffusion method. Filtrates of 57 staphylococci showed strong activity against *Listeria monocytogenes* Scott A, and 52 isolates also inhibited the growth of *Staphylococcus aureus* Newbould 305, a major causative agent of bovine mastitis in the United States. The plasmid profiles of staphylococci invariably included an 8-kb plasmid. Staphylococcal isolates were tested for the production of aureocins A70 and A53, 2 bacteriocins of coagulase-positive staphylococci known to be associated with 8-kb and 10.2-kb plasmids, respectively. The presence of the A70 or A53 bacteriocin gene was checked by PCR techniques using primers based on nucleotide sequences flanking the structural gene of each bacteriocin. Agarose gel analysis of amplified PCR products of plasmid templates from all 58 isolates showed only a 525-bp fragment corresponding to the structural gene of the bacteriocin aureocin A70. The results indicated that the apparently widespread association of A70-producing staphylococci with healthy cows in Brazil may be beneficial in controlling undesirable bacteria in dairy herds.

Key words: staphylococci from milk, antilisterial activity, bacteriocin

INTRODUCTION

Bacterial cells produce several types of substances that have inhibitory activity against other bacteria. These substances constitute a diverse class of molecules and have been identified, for example, as hydrogen peroxide, lactic acid, exotoxins, lytic enzymes, classical antibiotics, and bacteriocins (Jack et al., 1995). Bacteriocins are ribosomally synthesized antimicrobial peptides that are produced by many different bacterial species. The inhibitory activity is generally directed against the same or closely related species (Jack et al., 1995).

Bacteriocins have been found over the whole range of gram-negative and gram-positive species and have been isolated from a variety of habitats. Several bacteriocins have been extensively studied because there is growing interest in their potential application as food preservatives (Jack et al., 1995; Deegan et al., 2006). Nisin, which is produced by several strains of lactococci, is approved for food uses in more than 40 countries and has been used as a food preservative for over 50 yr (Chen and Hoover, 2003). Other bacteriocins from lactic acid bacteria such as pediocin and lactacin may also have applications as biopreservatives (O'Sullivan et al., 2002).

The growing interest in bacteriocins is also guided by their potential in therapeutic applications. The rise and spread of multidrug-resistant bacterial pathogens has motivated research for alternative antimicrobials to treat infectious diseases. Natural antimicrobial peptides such as bacteriocins may be valuable as supplemental agents in overcoming the growing problem of resistance to conventional antibiotics (Papagianni, 2003).

The antimicrobial spectrum of staphylococcal bacteriocins varies and may include species of the genera *Staphylococcus*, *Streptococcus*, *Bacillus*, *Clostridium*, *Corynebacterium*, and *Listeria*. Staphylococci were described in *Staph. aureus* isolated from human skin lesions belonging to phage group II type 71 (Barrow, 1963; Dajani and Wannamaker, 1969). Examples of these bacteriocins are produced by *Staph. aureus* strains C55 and UT007 (Warren et al., 1975; Rogolsky and Wiley,

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1977; Crupper et al., 1997). It was subsequently established that these 2 staphylococci are similar and that the bacteriocins structural genes were closely associated with the exfoliative toxin B determinant, which is located on the same plasmid. The staphylococci belong to the group of 2-component lantibiotics (Navaratna et al., 1998, 1999). Additional bacteriocins produced by strains of *Staph. aureus* include staphylococci 414 (Gagliano and Hinsdill, 1970), 462, and Bac1829 (Crupper and Iandolo, 1996), and the aureocins A53 and A70 (Giambiagi-Marval et al., 1990). Aureocins A53 and A70 are plasmid-mediated nonlantibiotic bacteriocins that were described in *Staph. aureus* isolated from pasteurized milk. The antimicrobial activity of aureocin A70 requires the presence of 4 unmodified peptides (Netz et al., 2001). Aureocin A53 is characterized by a rigid structure in aqueous solution that is markedly resistant to protease activity (Netz et al., 2002).

Several bacteriocins are produced by strains of *Staphylococcus epidermidis*. They include epidermin and its variant Val-1 Leu-6 epidermin (Sahl and Bierbaum, 1998), Pep5 (Sahl and Brandis, 1981; Sahl and Bierbaum, 1998), epicidin 280 (Heidrich et al., 1998), epilancin K (Van de Kamp et al., 1995), and the closely related epilancin 15X (Ekkelenkamp et al., 2005). Gallidermin (Leu-6 epidermin) produced by *Staphylococcus gallinarum* (Schnell et al., 1989) is also considered a natural variant of epidermin (Sahl and Bierbaum, 1998). All of these bacteriocins share a common feature of being elongated cationic type A lantibiotics and, with a few exceptions, their genetic determinants are carried on plasmids (Schnell et al., 1989; Van de Kamp et al., 1995; Ekkelenkamp et al., 2005). Two bacteriocins have been reported in *Staphylococcus warneri*, warnericin and nukacin ISK-1. The second is produced by *Staph. warneri* ISK-1 and has been characterized as a type A lantibiotic structurally similar to lactacin-481 (Sashihara et al., 2000; Minamikawa et al., 2005). Other staphylococci species that have been reported as bacteriocin producers include *Staphylococcus simulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis*, and *Staphylococcus arlettae* (Nascimento et al., 2005a).

In the present study, a collection of staphylococci recovered from the milk of healthy cows from several farms in Brazil was screened for bacteriocin production. The research focused on the activity spectrum of putative bacteriocins of staphylococcal isolates and applying PCR techniques to determine whether the bacteriocins detected were novel or similar to others described previously in the literature. Molecular techniques were also used to reduce the size of a bacteriocin plasmid and to evaluate its transferability to *Staphylococcus carnosus*, a food-grade species in the genus *Staphylococcus* that is used as a starter culture in fermented sausages.

MATERIALS AND METHODS

Source of Staphylococci and Culture Conditions

Staphylococci isolates ($n = 111$) were obtained from freshly drawn bovine milk samples from 41 dairy herds located in the southeast and northeast regions of Brazil. Milk samples were collected from individual cows just before milking and staphylococci were isolated on blood agar plates. They were identified as coagulase-positive (94) and coagulase-negative (17) according to colony and cellular characteristics, catalase production, and tube-coagulase test. The identity of 3 coagulase-positive staphylococci (numbers 48, 76, and 80) was further evaluated by the agglutination test for protein A and clumping factor (BactiStaph, Remel, Lenexa, KS).

In the genetic transformation experiments, host strains included *Staph. aureus* SA113 (ATCC 35556) and a commercial strain of *Staph. carnosus* (CS 299 Bactoform, Chr. Hansen Laboratories, Milwaukee, WI). All staphylococcal strains were cultured in tryptic soy broth (TSB, Becton, Dickinson and Co., Franklin Lakes, NJ) and stored under refrigeration conditions with monthly transfers. Long-term storage of cultures was at -80°C in TSB containing 20% (vol/vol) sterile glycerol.

Screening for Bacteriocin Production

Bacteriocin activity was evaluated by the agar-well diffusion method (Tagg and McGiven, 1971). The producer strains were grown overnight in TSB, and 50 μL of cell-free culture supernatants were transferred into wells cut in agar plates that were previously inoculated with selected indicator bacterial strains. After inoculation, the plates were stored for up to 16 h at 4°C , followed by incubation at 32°C for *Listeria* species or 37°C for *Staphylococcus* species. The presence of an inhibition zone indicated the production of antibacterial substances. The indicator strains included *Listeria monocytogenes* Scott A (LMS, ATCC 49594), *Listeria ivanovii* (LIV, ATCC 19119) and *Staph. aureus* Newbould 305 (SA305, ATCC 29740), a bovine mastitis agent frequently isolated in the United States (Guidry et al., 1998).

Bacteriocin activity was measured by the spot-on-the-lawn method (Henderson et al., 1992) following a 2-fold dilution with distilled water of cell-free supernatants of 18-h broth cultures. Five-microliter aliquots of samples were spotted on lawns seeded with LMS. The titer, in arbitrary units (AU) per milliliter, was defined as the reciprocal of the highest dilution still producing a visible inhibition zone.

Thermal stability of the putative bacteriocins in selected staphylococcal isolates was tested by heating cell-free supernatants for 10 min at 80 and 100°C and for 15 min at 121°C in the autoclave. Nonheated control samples were kept at 4°C. After heating, the samples were cooled, serially diluted, and tested for activity against LMS by the spot-on-the-lawn agar diffusion method.

Plasmid Isolation and Molecular Genetic Techniques

Large-scale isolation of plasmids was carried out according to a previously published protocol (Somkuti and Steinberg, 1988) and further purified by CsCl/ethidium bromide ultracentrifugation (Stougaard and Molin, 1983). Small-scale plasmid isolation was done by the rapid mini-prep method of O'Sullivan and Klaenhammer (1993). Staphylococcal cells were lysed by adding a 2 mg/mL lysostaphin (Sigma-Aldrich, St. Louis, MO) solution in 20 mM Tris (pH 8.2). Plasmid DNA was analyzed by agarose gel electrophoresis (AGE) in Tris-borate-EDTA buffer (0.089 M Tris base, 0.089 M boric acid, 0.002 M Na-EDTA, pH 8). The digestion of purified plasmids with *HincII*, *EcoRI*, *EcoRV*, and *PstI* restriction endonucleases was carried out as recommended by the manufacturer (New England Biolabs, Beverly, MA).

PCR Amplifications and 16S rDNA Analysis

Amplifications of known aureocin genes with specific oligonucleotides primers were made using as templates either total DNA recovered by the Prepman Ultra DNA isolation reagent (Applied Biosystems, Foster City, CA) or the isolated plasmids. The primers used were as follows: 5'-GATTAAACCTTATAATAGA-3' (forward) and 5'-CTAATAATAAAATATTAACAA-3' (reverse) for the aureocin A70 operon (*aurABCD*); 5'-GAAGT-TATGAAAACCTATA-3' (forward) and 5'-CATAAAA-CAAAGAACCAAGT-3' (reverse) for aureocin A53 (*aucA*); and 5'-GAGTGGAGAAACAGTAGT-3' (forward) and 5'-CTATTCTTCCCAATTTCAT-3' (reverse) for the replicon region of pRJ6, the original plasmid of aureocin A70-producing strain (Giambiagi-Marval et al., 1990). The primers for aureocins A70 and A53 were based on Nascimento et al. (2005a), and primers for pRJ6 replicon were designed according to the published partial sequence of the plasmid (Netz et al., 2001). The PCR reactions were carried out in a 50-μL volume containing 45 μL of Platinum PCR Supermix (Applied Biosystems), 2 μL of each primer, and 1 μL of DNA template. After the denaturing step of 3 min at 94°C, the protocol consisted of 35 amplification cycles: 5 s at 94°C, 30 s at 45°C, and 90 s at 72°C. The PCR products

were separated on 1.0% agarose gel electrophoresis and visualized under UV light after staining with ethidium bromide. An A70 aureocin-producing *Staphylococcus* culture (strain 4147, gift of M. C. Bastos, Federal University of Rio de Janeiro, Brazil) was used as a positive control.

Isolated genomic DNA was used as template for PCR with the eubacterial oligonucleotide primers EubA and EubB (Cottrell and Kirchman, 2000) for the amplification of 16S rRNA genes. The PCR products were cleaned with the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sequenced.

Nucleic acid sequencing of PCR products was performed using an ABI Prism 3730 DNA analyzer with ABI Prism Big Dye terminator cycle sequencing reagent (Perkin-Elmer, Wellesley, MA). Sequences were analyzed using Sequencer 4.2 (Gene Codes Corp., Ann Arbor, MI) and compared with sequences available in GenBank using the National Center for Biotechnology Information BLASTN search program (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Transformation of *Staph. aureus* and *Staph. carnosus*

Transfer of the bacteriocin plasmid to *Staph. aureus* SA113 and *Staph. carnosus* CS299 was performed by electroporation. The recipient bacteria were cultivated overnight in 5 mL of B2 medium (Schenk and Laddaga, 1992) incubated at 37°C. An overnight culture was used to inoculate 25 mL of B2 medium (1%, vol/vol) followed by incubation as above until reaching an optical density value at 660 nm of 0.5 to 0.6. The culture was chilled on ice for 15 min and the cells harvested by centrifugation at $10,000 \times g$ for 10 min at 4°C. The cells were washed 3 times with an equal volume of sterile water, washed once with 5 mL of 500 mM sucrose, 1 mM MgSO₄, and 1 mM HEPES buffer (SMH, pH 7.0), suspended in 2.5 mL of SMH buffer, and incubated at 20°C for 15 min. The cells were again centrifuged and resuspended in 1.0 mL of SMH buffer.

For electroporation, 20 to 25 μg of plasmid DNA was added to 100 μL of the cell suspension in a 0.2-cm cuvette. Using a BioRad Gene Pulser (Bio-Rad Laboratories, Richmond, CA), a single pulse was delivered at 2.5 kV, 100 Ω, and 25 μF, giving a field strength of 12.5 kV/cm. Immediately after pulsing, 900 μL of SMMP (0.5 M sucrose, 20 mM maleic acid, 20 mM MgCl₂, 3.5% Difco Penassay broth, pH 6.5) was added to the cuvette (Augustin and Gotz, 1990), the transformation mixture was transferred to a sterile tube and incubated for 2 h at 37°C. After incubation, 100-μL aliquots were spread on plates prepared as described below, and incubated overnight at 37°C. Selected colonies were checked

Figure 1. Plasmid profiles of staphylococcal isolates from bovine milk (lanes 1–5) and supercoiled molecular markers (lane 6).

for antibacterial activity against LMS by the agar-well diffusion method.

Because a readily detectable antibiotic marker was not available, the selection of genetic transformants was based on the assumption that uptake of the bacteriocin plasmid would also result in the acquisition of the immunity gene. Thus, the selective medium for transformed bacteria was prepared with a crude aureocin preparation, by removing the cells by centrifugation ($10,000 \times g$, 4°C) from the overnight culture (TSB) of a producer strain, adjusting the supernatant to pH 7.0 with 5 M NaOH, adding agar at 1.5% (wt/vol), and autoclaving the medium at 121°C for 17 min before pouring plates.

RESULTS AND DISCUSSION

The results of the screening for antibacterial activity of the 111 isolates of staphylococci are summarized in Table 1. A total of 57 (51.4%) isolates showed antimicrobial activity and, with the exception of 5 isolates, all strains showing activity against LMS also inhibited SA305, but only 43 isolates (38.7%) had inhibitory activity against LIV.

Among the staphylococcal isolates, strains 48, 76, and 80 produced the largest inhibition zones against indicator bacteria (LMS, SA305, and LIV) and were selected for further studies. All 3 strains agglutinated with antiserum for protein A and clumping factor and were designated as *Staph. aureus*. The identity of strain 80 was further confirmed by PCR amplification of 16S ribosomal DNA. Subsequent comparison of sequences with the BLAST database showed 100% identity with *Staph. aureus*.

The antibacterial activity of strains 48, 76, and 80 after 18 h of growth in TSB medium was estimated at 1,600 AU/mL. Heating at 80, 100, or 121°C had no effect on the antibacterial activity. Inhibition zones produced following heat treatments were comparable to those of control samples kept at 4°C , indicating that the putative bacteriocins produced were heat-resistant.

Plasmids were recovered from 52 isolates showing activity against *Listeria* or SA305. These strains had a varying number of resident plasmids (1 to 3), with sizes estimated at 4, 8, and 12 kb (Figure 1). The presence of an approximately 8-kb plasmid was a common feature in all isolates. The 3 strains selected for further studies (strains 48, 76, and 80) each had only the 8-kb plasmid, which were correspondingly designated as p48, p76, and p80. A similar-size plasmid that carries the genes of the aureocin A70 (pRJ6) was described in staphylococcal strains isolated from bovine milk in Brazil by Giambiagi-Marval et al. (1990).

Several staphylococcal bacteriocin genes have been found in plasmids with lengths varying from 8 to >50 kb (Sahl and Brandis, 1981; Crupper and Iandolo, 1996; Crupper et al., 1997; Heidrich et al., 1998; Navaratna et al., 1998, 1999). The A70 and A53 aureocin plasmids originally described by Giambiagi-Marval et al. (1990) were reported to have antilisterial activity by Oliveira et al. (1998).

Table 1. Inhibition of *Listeria monocytogenes* Scott A (LMS), *Listeria ivanovii* (LIV), and *Staphylococcus aureus* Newbold 305 (SA305) by staphylococcal isolates from bovine milk

Group	No. tested	LMS	LIV	SA305
Coagulase-positive staphylococci	94	54	43	49
Coagulase-negative staphylococci	17	3	0	3
Total	111	57	43	52

Figure 2. Products of PCR generated with primers flanking the *aurCABCD* operon (lane 1) and the replication gene (lane 3) in *Staphylococcus aureus* isolate 48; 100-bp (lane 2) and 500-bp (lane 4) molecular weight markers are shown.

Digestion with restriction enzymes showed that, similar to the published map of pRJ6 (Giambiagi-Marval et al., 1990), p76 and p80 had a single site for *EcoRI* and *PstI* and 2 sites for *EcoRV*. The restriction fragments produced with *EcoRV* were similar in size (~2.5 kb and 5.5 kb) to those described for pRJ6 (Giambiagi-Marval et al., 1990; Netz et al., 2001). Plasmid p48 also had a single site for *EcoRI* and *PstI* but had an extra site for *EcoRV*, resulting in fragments of approximately 2.1, 2.2, and 3.7 kb. The number of sites for *HincII* was also different in p76 and p80 and p48. The first 2 had 4 *HincII* sites, resulting in fragments of approximately 0.4, 0.8, 1.4, and 5.4 kb, whereas p48 had 5 sites and generated fragments of approximately 0.4, 0.8, 1.3, 1.4, and 4.1 kb.

All 57 staphylococcal isolates showing antilisterial or anti-SA305 activity yielded a PCR product of approximately 525 bp with primers specific for the A70 structural gene (Figure 2). The amplified product corresponded to the *aurCABCD* aureocin operon of A70 (Netz et al., 2001) with either genomic DNA or isolated plasmid used as the template.

The PCR assays with DNA used as template from p48, p76, p80, and employing specific primers flanking the replication gene of pRJ6 were also conducted. The PCR product obtained was approximately 857 bp

with p48, p76, and p80, which is the expected size of the pRJ6 replicon (Figure 1). These results indicated that the 8-kb plasmid found in the 3 staphylococcal strains may be similar or related to pRJ6, which was originally described to carry the genes of aureocin A70 (Giambiagi-Marval et al., 1990).

The uptake of p48, p76, and p80 by *Staph. aureus* SA113 (SA113; ATCC 35556) was accomplished by electroporation. After plating out the transformation mixtures, colonies were randomly picked and cultured overnight in TSB. Cell-free culture supernatants were checked for inhibition of LMS by the agar-well diffusion method. A total of 376, 99, and 75 colonies were checked against LMS after transformation of SA113 with p48, p76, and p80, respectively. The number of colonies showing inhibition zones after transformation with p48, p76, and p80 was 2, 6, and 5, respectively. The presence of an approximately 8-kb plasmid was confirmed in all isolates of transformed SA113. The digestion of purified plasmid preparations of p48, p76, and p80 from SA113 transformants with *EcoRV* and *HincII* yielded the expected pattern of restriction fragments. In addition, PCR products obtained with primers specific for the aureocin A70 structural gene and for the replicon of pRJ6 (the original plasmid of aureocin A70) were also identified.

According to the published map of pRJ6 (Giambiagi-Marval et al., 1990) and genetic analyses of the 6,332-bp fragment of the plasmid (Netz et al., 2001), the 5.5-kb fragment obtained by *EcoRV* digestion would include the aureocin A70 structural and immunity genes and encode the transport system in addition to the replicon of the plasmid. Thus, the plasmid p80 isolated from SA113 was digested with *EcoRV* and ligated to yield the smaller p80a (~5.5 kb), which was transformed into SA113 and evaluated for activity against LMS. Plasmid analysis of 22 colonies with antilisterial activity confirmed the presence of the 5.5-kb plasmid in 15 isolates, whereas 7 isolates harbored the apparently religated 8-kb p80 plasmid. However, the inhibition zones produced by strains carrying the reduced-size plasmid were smaller than those produced by the bacteria carrying the p80 parent plasmid (data not shown). These results indicated that some functions related to the production or transport of the bacteriocin or plasmid replication may be located in the excised fragment.

Transformation of the commercial strain *Staph. carnosus* CS299 was attempted with purified p48 and p80 plasmids extracted from their original SA48 and SA80 strains or from the transformants SA113/p48 and SA113/p80. *Staphylococcus carnosus* was selected for the transformation experiments because it is a non-pathogenic, food-grade staphylococcus used in the fermentation of sausages (Schleifer and Fischer, 1982). As

a starter culture, *Staph. carnosus* enhances the flavor and contributes to the reddening reaction in sausages (Hammes et al., 1995; Leroy et al., 2006); it has been used as a host for gene cloning and protein production (Gotz, 1990) in studies on bacteriocin gene expression (Schnell et al., 1992) and for surface display of heterologous gene products (Wernéus et al., 2002).

Several attempts were also made to transform *Staph. carnosus* CS299 with the plasmids isolated from strains 48 and 80. Although culture filtrates of *Staph. carnosus* isolates lacked antilisterial activity, transformants obtained with p48 contained an 8-kb plasmid in addition to the 4-kb resident plasmid that in some isolates indicated loss of molecular mass possibly due to deletion mutation events (data not shown). Further, PCR analysis of the isolated 8-kb plasmid with primers to the aureocin A70 structural gene and the replicon of pRJ6 yielded amplification products with sizes of approximately 525 and 857 bp, which are the expected sizes of the aureocin A70 structural gene and the replicon of p48, respectively. This plasmid preparation from *Staph. carnosus* CS299 was also used successfully to transform SA113. The 40 colonies of SA113 transformants checked against LMS all produced inhibition zones. These results indicated that the bacteriocin plasmid p48 was transferred to *Staph. carnosus* CS299 but antilisterial activity was not expressed in this host. The failure of expression may be related to the lack of secretion of the bacteriocin by *Staph. carnosus* cells or to mechanisms that regulate the production of the bacteriocin. Although the expression of antibacterial activity of cloned lantibiotic genes in *Staph. carnosus* has been shown before (Schnell et al., 1992; Heidrich et al., 1998), attempts to transfer the whole bacteriocin plasmid of *Staph. aureus* C55 encoding a 2-component lantibiotic were unsuccessful (Navaratna et al., 1998).

The present investigation showed widespread production of bacteriocins by staphylococci isolated from bovine milk. Antimicrobial activity was mediated by an 8-kb plasmid, and the putative bacteriocin appeared to be identical to or a natural variant of aureocin A70. The production of aureocin A70 is plasmid mediated and was described by Giambiagi-Marval et al. (1990) in *Staph. aureus* isolated from milk samples in Brazil. Subsequently, it was reported in *Staph. aureus* isolated from Argentinian and Brazilian dairy herds (Nascimento et al., 2002, 2005a) and also in CNS recovered from cases of bovine subclinical mastitis (Nascimento et al., 2005b).

The bacteriocin plasmid was successfully transferred to SA113, a plasmid-free strain that is commonly used as recipient for electroporation of staphylococcus plasmids (Lee, 1995). The transformed bacteria expressed the inhibitory activity against LMS and produced in-

hibition zones similar in size as the original cultures. The plasmid was also transferable by electroporation to *Staph. carnosus*, but inhibitory activity could not be demonstrated against LMS.

Genes that regulate the expression of lantibiotics (Siezen et al., 1996) may also be necessary for the expression of the aureocin A70. It was found that when p80 from SA113 transformants was digested with *EcoRV* and religated to the reduced size p80a (5.5 kb), smaller inhibition zones were produced against *L. monocytogenes* than with the whole plasmid. The approximately 2.5-kb excised fragment possibly encoded some of the functions related to bacteriocin production or secretion.

In addition to the antilisterial effect that may help in controlling outbreaks of milk-borne listeriosis, another noteworthy finding in this study was the anti-SA305 activity of aureocin-producing staphylococcal isolates that naturally occur in many milk samples collected from Brazilian dairy herds. Staphylococci are responsible for approximately 30% of bovine mastitis outbreaks (Sutra and Poutrel, 1994), and *Staph. aureus* SA305 is 1 of the 3 major serotypes of staphylococci that account for 100% of US and 98% of European cases of intramammary infections in cows (Guidry et al., 1998). Because the prevention of bovine mastitis by vaccine therapy has had limited success and strategies involving the production of recombinant lysostaphin by genetically enhanced cows are still in development (Wall et al., 2005), consideration should be given to further research on aureocin-producing nonpathogenic staphylococci to evaluate their potential as natural antimicrobial agents for controlling the growth of infectious staphylococci involved in the outbreaks of mastitis on dairy farms.

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