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Hyicin 3682, a bioactive peptide produced by *Staphylococcus hyicus* 3682 with potential applications for food preservation

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Abstract

Bacteriocins are peptides produced by bacteria and having inhibitory activity against other bacteria. Many of these substances may be useful as antibacterial agents for practical applications. In this study, 21 *Staphylococcus* spp. isolated from pigs, dogs and bovine milk in different states of Brazil were investigated for staphylococcin production. Hyicin 3682, a bacteriocin produced by one such strain, inhibited almost all strains tested, including *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*. PCR experiments showed that hyicin 3682 is lantibiotic-related, but not identical, to both epidermin and Bsa. The maximum production of hyicin 3682 (6,400 AU/ml) was observed after 24 h of growth in BHI medium at 37 °C. Hyicin 3682 proved to be a cationic, small antimicrobial peptide with a molecular mass of 2,139 Da. It exhibited resistance to low pH and to heating at 65 °C, and partial sensitivity to proteolytic enzymes. Taken together, these results suggest that hyicin 3682, the first bacteriocin characterized in *Staphylococcus hyicus*, has potential biotechnological applications as a food preservative. Moreover, hyicin 3682 was able to inhibit its producer strain, suggesting that an effective immune system for specific protection against hyicin 3682 is not found in its producer strain, a characteristic not described thus far for other staphylococcins.

Keywords: Bacteriocin; *Staphylococcus hyicus*; Bioactive peptide; Hyicin 3682; Food preservation

1. Introduction

Bioactive peptides are produced by a wide range of organisms, including bacteria, and have a positive impact on body functions and conditions that may influence health (Korhonen, 2009). Bacteriocins are ribosomally synthesized peptides produced by bacteria, possessing inhibitory activity against other bacteria. Bacteriocin-producing strains have developed a protection system against their own bacteriocins, termed immunity. Genes encoding bacteriocin production and immunity are often located on plasmids, although they can be found on the bacterial chromosome as well (Heng et al., 2007). Several bacteriocins of Gram-positive bacteria display bactericidal activity with fairly broad inhibitory spectra and may have practical applications as antibacterial agents (Hata et al., 2010).

Bacteriocins produced by Gram-positive bacteria and belonging to classes I (lantibiotics) and II are currently being widely investigated because of their frequent occurrence and their potential applications in the food industry and in clinical medicine (Heng et al., 2007; Bastos et al., 2009). At present, only two bacteriocins have been approved as preservatives in food: nisin, which is a class I bacteriocin, and pediocin PA-1, which is a class II bacteriocin (Cotter et al., 2005).

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Current food preservation methods based on potentially toxic chemical compounds are being questioned by consumers (Gálvez et al., 2007; Sobrino-López and Martín-Belloso, 2008; Keymanesh et al., 2009). The discovery of new bacteriocins, which are considered natural substances, combined with the development of methods for their application, might, therefore, be beneficial in food applications (Deegan et al., 2006). These antimicrobial peptides are not merely preservatives, but often influence the quality and flavor of the food product as well (Keymanesh et al., 2009).

Staphylococcins are bacteriocins produced by strains belonging to the genus *Staphylococcus*. Since the beginning of our studies, new staphylococcins have been characterized by our group; these include aureocins A53, A70 and 4185 (Bastos et al., 2009; Ceotto et al., 2010). Aureocin A70 was the first bacteriocin to be described and is composed of four related small peptides, which are encoded by the *aurABCD* operon found on pRJ6 (Giambiagi-deMarval et al., 1990; Netz et al., 2001). This bacteriocin has been re-isolated many times from either different strains of *Staphylococcus aureus* or coagulase-negative *Staphylococcus* (CoNS) (Bastos et al., 2009).

Epidermin and its variants seem to be the most frequently produced lantibiotics in the group of CoNS, as they have been re-isolated from many strains of *Staphylococcus epidermidis* and also from other staphylococcal species (Bastos et al., 2009). The structural gene of epidermin, *epiA*, is encoded on the 54-kb plasmid pTü32 (Augustin et al., 1992).

In 2010, genome sequencing revealed the presence of genes apparently encoding a lantibiotic related to epidermin and designated Bsa among several clinical isolates of *S. aureus*, in particular, those associated with community-acquired methicillin-resistant *S. aureus* infections (Daly et al., 2010).

Many bacterial species are inhibited by staphylococcins, including bacterial pathogens, which make these peptides important tools in the food industry (Bastos et al., 2009). Therefore, the aim of this study was to detect new bacteriocins produced by *Staphylococcus* spp. strains of animal origin that could have potential applications in food preservation.

2. Materials and methods

2.1. Bacterial strains and growth conditions

In the present study, 21 *Staphylococcus* spp. were investigated for antimicrobial substance production. These bacterial strains were isolated from pigs, dogs and bovine milk in different states of Brazil.

S. aureus and *S. epidermidis* strains from previous studies were also used either as producers or as indicators in inhibition assays and are listed in Table 1. *Staphylococcus* spp. were grown in TSB (Difco) or BHI (Difco) at 37 °C for 18 h. TSB was used to grow strains for DNA isolation and BHI was used in all bacteriocin assays.

The bacteria used as indicators in analysis of the inhibitory spectrum belonged to different species of either lactic acid bacteria (LAB) or food-borne pathogens and spoilage bacteria. They were grown in BHI medium at 37 °C, except for LAB which were cultivated in the appropriate medium, MRS (Man, Rogosa and Sharpe; Difco) at 28 °C.

Table 1
Staphylococcus spp. previously described and used in this study.

Strain	Relevant features	Reference/source
Staphylococcus aureus		
A70	Bac$^+$ (aureocin A70), Imm$^+$, pRJ6 (7.9 kb)	Giambiagi-deMarval et al. (1990)
A53	Bac$^+$ (aureocin A53), Imm$^+$, pRJ9 (10.4 kb)	Giambiagi-deMarval et al. (1990)
COL1881	a COL derivative, Bsa$^+$, Imm$^+$	Daly et al. (2010)
Staphylococcus epidermidis		
Tü3298	Bac$^+$ (epidermin), Imm$^+$, pTü32 (54 kb)	Augustin et al. (1992)

Bac, bacteriocin; Imm, immunity.

Bacteria were stored in their appropriate medium with 40% glycerol (w/v) at −20 °C until required. For preparation of either solid or soft-agar media, agar was added at 1.5% (w/v) or 0.6% (w/v), respectively.

2.2. Assay for antimicrobial substance(AMS) production

This assay was performed as described previously by Ceotto et al. (2010) in BHI agar plates, using *Corynebacterium fimi* NCTC7547 as the target microorganism.

2.3. Identification of the strains

Only staphylococcal strains that exhibited AMS production were identified at the species level, using conventional biochemical tests (Bannerman and Peacock, 2007) and a commercial kit for identification (API Staph, bioMérieux, Marcy l'Etoile, France). The identification of *Staphylococcus hyicus* 3682 was confirmed by PCR amplification of 16S rDNA using primers 11F (5′ TAA CAC ATG CAA GTC GAA CG 3′) and 5R (5′ GGT TAC CTT GTT ACG ACT T 3′). The reactions were made in a 50 µl final volume containing: (i) 50 pmol of each primer; (ii) 5 ng of genomic DNA; (iii) 2.5 mM concentration of each deoxyribonucleoside triphosphate; (iv) 2.5 U of *Taq* DNA-polymerase (Fermentas); (v) 5 mM $MgCl_2$; and (vi) 1x reaction buffer (Fermentas). The thermal cycling consisted of an initial denaturation step at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 58 °C for 30 s (annealing) and 72 °C for 90 s, and a final extension step at 72 °C for 5 min. The amplicon generated was sequenced at the Norwegian University of Life Science and the sequence was compared with others deposited in the GenBank database using the BLASTn software.

2.4. Effects of proteolytic enzymes and NaOH on AMS activity on solid medium

The effects of trypsin (Sigma), proteinase K (Sigma), protease XXIII (Sigma), and 0.2 M NaOH on AMS activity were initially determined on solid medium by methods described previously (Giambiagi-deMarval et al., 1990).