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SHORT COMMUNICATION

Evidence for production of a bacteriocin-like substance by *Staphylococcus pseudintermedius*, inhibitory to *Staphylococcus aureus* from foods

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This study assessed the production of a bacteriocin-like substance by *Staphylococcus pseudintermedius* S28, and evaluates its inhibitory effect against isolates of *S. aureus* from foods. All indicator isolates were sensitive to the substance produced from *S. pseudintermedius* S28, showing growth inhibition zones ranging from 14.2 to 28.3 mm. The inhibitory substance has no effect against the producer strain. The inhibitory substance was affected by proteolytic enzymes, while glycolytic and lipolytic enzymes had no effect, suggesting that the active substance could be considered as a bacteriocin-like substance. From these results, *S. pseudintermedius* S28 could be an interesting producer of a bacteriocin-like substance capable of strongly inhibiting *S. aureus*.

Keywords: *Staphylococcus pseudintermedius*; bacteriocin; anti-staphylococcal activity

1. Introduction

Bacteriocins are ribossomally synthesised antimicrobial peptides produced by some bacteria. In contrast to Gram negative bacteria, bacteriocins from Gram positive bacteria present a wide spectrum of inhibitory activity (Gálvez, Abrioul, López, & Omar, 2007).

In *Staphylococcus* genera, the specie *Staphylococcus aureus* are known as the most important producers of bacteriocins (Kim et al., 2010). However, the production of bacteriocins by other coagulase-negative staphylococci has already been described, including rumen staphylococci (Lauková & Mareková, 1993) and the meat starter culture *S. xylosus* (Lauková, Simonivá, & Strompfová, 2010). Bacteriocins from staphylococci have revealed some efficacy for inhibiting food-related bacteria being known as potential antimicrobial substances to use in food preservation (Galvéz et al., 2007).

The coagulase positive members forming the *S. intermedius* – group (SIG), i.e. *S. intermedius*, *S. pseudintermedius* and *S. delphini*, are the most clinically relevant staphylococci in veterinary medicine (Weese & van Duijkeren, 2010). Although members of SIG have been the subject of numerous studies regarding their role in pathogenesis of animal infectious and resistance to antimicrobials (Fazakerley, Williams, Carter,

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McEwan, & Nuttall, 2010), there is a lack of studies emphasising the production of bacteriocin-like substances by these species. The aim of this study was to evaluate the antimicrobial activity produced by a strain of *S. pseudintermedius*, inhibitory to *S. aureus* from foods.

2. Results and discussion

In previous analysis aiming to assess the production of inhibitory substance from 18 isolates of *S. pseudintermedius*, the isolate S28 showed remarkable efficacy to inhibit *S. aureus* forming growth inhibition zones of up to 45 mm diameter. In this study, we investigated further the efficacy of the substance produced by *S. pseudintermedius* S28 in inhibiting some isolates of *S. aureus* from foods. All tested isolates of *S. aureus* were sensitive to the substance produced by *S. pseudintermedius* S28 showing growth inhibition zones with an average diameter ranging from 14.2 to 28.3 mm (Table 1). Still, the inhibitory substance had no effect against the producer strain, which is one of the most important properties of bacteriocins (Cladera-Olivera, Caron, & Brandelli, 2004).

Table 1. Inhibition zones (mm) obtained with *S. pseudintermedius* S28 against strains *S. aureus* isolated from foods by the agar spot-on-lawn assay.

Indicator strain	Growth inhibition zones (\pm standard deviation)
<i>S. aureus</i> QR1-1	18.6 (\pm 2.0)
<i>S. aureus</i> QR2-1	14.2 (\pm 1.3)
<i>S. aureus</i> QR2-2	18.2 (\pm 2.3)
<i>S. aureus</i> QR2-4	17.4 (\pm 2.3)
<i>S. aureus</i> QR2-5	20.1 (\pm 2.1)
<i>S. aureus</i> QR2-6	21.2 (\pm 3.1)
<i>S. aureus</i> QR2-7	26.3 (\pm 3.3)
<i>S. aureus</i> QR3-3	17.4 (\pm 2.1)
<i>S. aureus</i> QR3-4	17.2 (\pm 1.9)
<i>S. aureus</i> QR3-5	18.1 (\pm 1.5)
<i>S. aureus</i> QR3-7	19.8 (\pm 2.1)
<i>S. aureus</i> QR6-1	20.7 (\pm 2.0)
<i>S. aureus</i> QR6-2	14.5 (\pm 1.2)
<i>S. aureus</i> QR6-4	25.1 (\pm 2.9)
<i>S. aureus</i> QR6-5	17.6 (\pm 1.8)
<i>S. aureus</i> QR6-6	19.2 (\pm 2.1)
<i>S. aureus</i> QR6-7	16.7 (\pm 1.9)
<i>S. aureus</i> QR6-8	23.1 (\pm 2.3)
<i>S. aureus</i> QR6-9	17.4 (\pm 1.8)
<i>S. aureus</i> QR6-10	18.3 (\pm 2.1)
<i>S. aureus</i> QR7-1	18.6 (\pm 1.8)
<i>S. aureus</i> QR7-2	17.2 (\pm 2.1)
<i>S. aureus</i> QR7-3	19.9 (\pm 2.8)
<i>S. aureus</i> QR7-4	16.7 (\pm 1.9)
<i>S. aureus</i> QR7-5	28.3 (\pm 3.1)
<i>S. aureus</i> QR7-8	15.2 (\pm 1.6)
<i>S. aureus</i> QR10-1	14.6 (\pm 1.3)
<i>S. aureus</i> QR10-2	18.5 (\pm 2.2)
<i>S. aureus</i> QR10-3	17.4 (\pm 1.3)
<i>S. aureus</i> QR10-4	15.1 (\pm 1.5)

The inhibitory substance produced by *S. pseudintermedius* S28 was affected by proteolytic enzymes trypsin and proteinase K, while glycolytic and lipolytic enzymes had no effect on the inhibitory activity. The growth of all test indicator strains around the area of the agar, where crystals of proteolytic enzymes were absorbed, indicates the proteinaceous nature of the substance produced from *S. pseudintermedius* S28. These findings also exclude the possibility of bacteriophage action (infection), since bacteriophage is greatly resistant to trypsin (Jack, Tagg, & Ray, 1999). Konisky (1982) defined bacteriocin as 'protein or protein complexes not active to the producer strain', and our results showed that the inhibitory substance produced by *S. pseudintermedius* S28 for this definition could thus be considered as a bacteriocin-like inhibitory substance.

Several bacteria have been previously identified as producers of bacteriocins, including *Staphylococcus* species, such as *S. epidermidis* producing epidermin (Fontana, Bastos, & Brandelli, 2006), *S. simulans* producing nukacin 3299 (Ceotto, Holo, & Costa, 2010), *S. hominis* producing hominacin (Kim et al., 2010), *S. equorum* producing micrococin P₁ (Carnio et al., 2000), *S. hyicus* producing hyicin 3682 (Fagundes et al., 2011) and *S. aureus* producing aureocin A70 (Netz et al., 2001).

To the best of our knowledge, this is the first report about the production of a bacteriocin-like substance by *S. pseudintermedius* inhibitory to *S. aureus* from foods. These findings encourage further researches on the purification and characterisation (molecular weight and protein sequencing) of the bacteriocin-like substance from *S. pseudintermedius* S28, and its inhibitory effect towards other spoilage and pathogenic food-related bacteria, besides the investigation of its mode of action on sensitive bacteria.

3. Conclusion

S. pseudintermedius S28 produced a bacteriocin-like substance with strong inhibitory effect toward several isolates of *S. aureus* isolated from foods.

Supplementary material

Experimental details relating to this article are available online.

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