Staphylococcus aureus is an important pathogen associated with food poisoning and one of the most prevalent agents of mastitis in cattle. In dairy products, the presence of S. aureus is usually associated with contamination of raw milk from animals with subclinical mastitis, or with post-pasteurization contamination due to improper handling of the product. Heat treatment of milk does not ensure the absence of the enterotoxins produced by numerous staphylococcal strains since they are heat stable. Bacteriophages and phage-encoded lytic proteins (endolysins and virion-associated peptidoglycan hydrolases (VAPGHs)) are a promising approach in the biocontrol of pathogens since they act as antimicrobial agents due to their ability to lyse Gram positive bacteria on contact. In this work, our aim was to enhance the antimicrobial activity of the VAPGH HydH5, encoded by the S. aureus bacteriophage vB_SauS-phiIPLA88, against S. aureus by domain swapping using the bacteriocin lysostaphin. Possible synergistic effects between HydH5 and the endolysin LysH5, encoded by the same phage, were also tested to explore alternative enzymic-based strategies to fight against S. aureus infections.

**RESULTS**

Two single domains, CHAP and LYZ2, and four deletion constructs were generated from HydH5 (Fig.1). We also proceeded to determine whether the addition of the lysostaphin binding domain SH3b might increase the lytic activity of the full-length HydH5 and the CHAP domain. The lysostaphin binding domain SH3b was fused to the full-length HydH5 protein, resulting in a protein with two catalytic domains and one cell wall binding (CBW) domain (HydH5SH3b). A second construct was obtained by fusion of the CHAP156 domain to the lysostaphin SH3b domain (CHAPSH3b). Finally, the full-length lysostaphin and HydH5 were fused in order to obtain a protein with three catalytic domains and a CBW domain (HydH5LysH5).

Fusion to lysostaphin sequences increases HydH5 and CHAP lytic activities

As shown in Fig. 2A, all proteins created a single zone of clearing in the zymogram assay, consistent with the predicted molecular mass and positions of the nickel affinity purified proteins in the SDS-PAGE. In the plate lysis assay (Fig. 2B) all the fusion proteins showed significantly greater activity than the parental protein HydH5, even against the methicillin-resistant (MRSA) S. aureus N315 strain. Of note, fusion of SH3b to the CHAP domain increased CHAP activity 64-fold (Fig. 2B).

In the turbidity reduction assay (Fig. 2C), the fusion proteins HydH5LysH5 and HydH5SH3b (1 µM) showed 1.5- and 1.7-fold higher activity than the parental protein HydH5, respectively. HydH5LysH5 had 1.5-fold higher activity than HydH5SH3b. CHAPSH3b, with an activity 4.8-fold greater than the CHAP domain, showed the highest lytic activity obtained from HydH5 fusions.

**CONCLUSIONS**

The lytic activity of HydH5 and its derivative fusions is specific for staphylococci

All proteins were active against S. aureus and Staphylococcus epidermidis strains but no other genus was lysed at detectable levels (data not shown). In general, S. aureus strains were more sensitive to lysis than S. epidermidis strains. Within the S. aureus strains, bovine strains were more sensitive than clinical strains. This could be due to the fact that bovine strains and the phage from which HydH5 originated have been isolated from the dairy environment.

The novel chimera proteins generated by combination between HydH5 and lysostaphin have improved lytic activity against S. aureus, including MRSA N315 and S. epidermidis. On the other hand, the effectiveness of HydH5 and its derivative fusions when used in combination with LysH5, another dairy derived anti-staphylococcal protein, were remarkable. We expect that these constructs will provide new weapons to combat multidrug-resistant S. aureus infections in both dairy and clinical environments.