



Killing and lysis of *Staphylococcus aureus* and other staphylococci by an endolysin

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Introduction

The use of endolysins for the treatment of skin infections or decontamination may circumvent antibiotic resistance. Micreos has developed an endolysin as a potential antibacterial compound. Staphefekt.SA100™ is a chimeric lysin featuring endopeptidase and putative amidase activities. The aim of this study was to determine the species specificity of Staphefekt.SA100™, the killing efficiency and effective concentration against several staphylococcal species.

Materials and methods

■ Twenty clinical relevant strains belonging to 15 species were tested (Table 1).

■ Staphefekt.SA100™ was produced in *E. coli* with recombinant technologies, and partially purified.

■ Endolysin was tested for bacterial lysis. The OD₆₀₀ was measured every 3 min during 30 min. Bacteria from a ON MHB culture were diluted 20-fold and grown to approximately 0.4 OD₆₀₀, collected and resuspended in PBS. The experiments were performed twice independently starting from blood agar plates.

■ Bacteria from an ON MHB culture were diluted 20-fold and grown to 0.3 OD₆₀₀ in fresh MHB. The bacteria were suspended in PBS. A total of 100 µl of this suspension was added to 9.9 mL of pre-warmed MHB resulting in 5x10⁵-5x10⁶ colony forming units (CFUs/mL). The suspension contained 30 µg/mL endolysin. Immediately after inoculation a sample was taken (0 h) for CFU determination. CFUs were also determined after 1, 3, 6 and 24 h.

Experiments were performed at least twice.

Results

■ All *Staphylococcus aureus* strains could be lysed by the endolysin (Table 1). A concentration of 30 µg/mL was still considered to be effective (example in Fig. 1).

■ *Staphylococcus pseudointermedius*, and *Staphylococcus hyicus* also showed a good lysis, whereas lysis of *Staphylococcus capitis* and *Staphylococcus hominis* was less. Lysis of *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* was hardly observed and was absent for *Staphylococcus lugdunensis*.

■ For the other species tested lysis could not be detected (Table 1).

■ For all *S. aureus* isolates excellent killing (>100x) was observed within 6 h (Figure 2). However, all isolates showed regrowth after 24 h.

■ Moderate killing (100-300x) was observed for *S. pseudointermedius* and *S. hyicus*. The other staphylococci were not killed (data not shown).

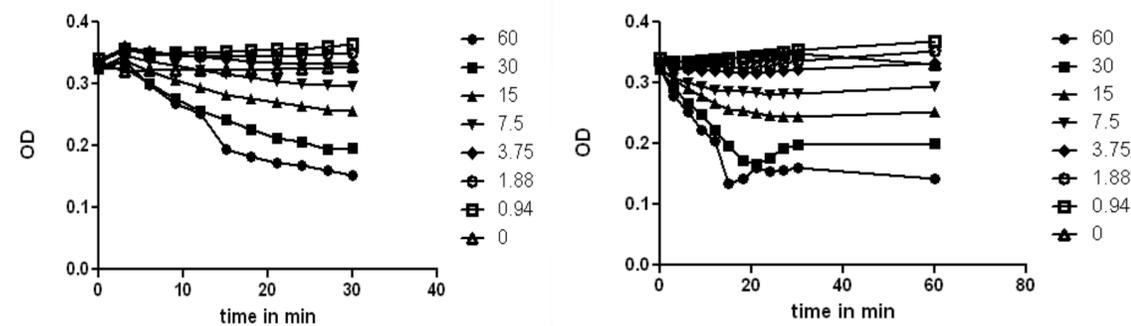


Figure 1. Lysis of *S. aureus* 5 by different endolysin concentrations (µg/mL) as indicated at the right of the figure.

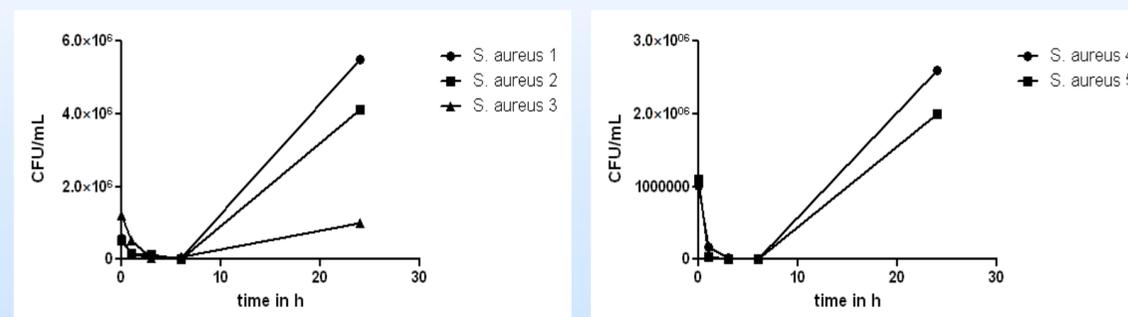


Figure 2. Killing curves for the *S. aureus* isolates.

Table 1. Lysis results for the tested strains after 30 min and 60 µg/mL endolysin.

| species | remarks | result lysis (%) |
|---|---------------------------|------------------|
| <i>Staphylococcus aureus</i> | Hospital-associated MRSA | 50 |
| <i>Staphylococcus aureus</i> | Community-associated MRSA | 60 |
| <i>Staphylococcus aureus</i> | Livestock-associated MRSA | 70 |
| <i>Staphylococcus aureus</i> | methicillin susceptible | 50 |
| <i>Staphylococcus aureus</i> | methicillin resistant | 0 |
| <i>Staphylococcus epidermidis</i> | methicillin susceptible | 10 |
| <i>Staphylococcus epidermidis</i> | methicillin resistant | 0 |
| <i>Staphylococcus haemolyticus</i> | | 10 |
| <i>Staphylococcus hominis</i> | | 20 |
| <i>Staphylococcus pseudointermedius</i> | | 40 |
| <i>Staphylococcus hyicus</i> | | 40 |
| <i>Staphylococcus capitis</i> | | 30 |
| <i>Staphylococcus lugdunensis</i> | | 0 |
| <i>Streptococcus pyogenes</i> | | 0 |
| <i>Streptococcus agalactiae</i> | | 0 |
| <i>Enterococcus faecalis</i> | | 0 |
| <i>Enterococcus faecium</i> | | 0 |
| <i>Escherichia coli</i> | | 0 |
| <i>Pseudomonas aeruginosa</i> | | 0 |
| <i>Acinetobacter spp.</i> | | 0 |

Conclusion

Efficient killing of different *S. aureus* strains is obtained by using 30 µg/mL endolysin. Staphylococcal species belonging to the normal human commensal flora as well other species are not affected. Topological application for *S. aureus* skin infections would leave the skin flora intact. The activity against *S. pseudointermedius* and *S. hyicus* may be useful in the treatment of companion animals and pigs since these species are pathogens for these animals, respectively.