

P001 Substrate specificity and mutational analysis of the glycyl-glycine activity of lysostaphin (32217)
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Lysostaphin which was first described in 1964 is a promising antimicrobial peptide that has activity against MRSA. Alanine mutagenesis was used to investigate the role of seven conserved residues, located in the endopeptidase domain, in the anti-staphylococcal activity of lysostaphin. Five of the alanine mutants abolished the killing activity of the purified lysostaphin mutant proteins in a turbidity assay, and the endopeptidase activity in a novel FRET assay. All five of the inactive, mutant proteins were able to protect *S.aureus* cells from killing by lysostaphin and thus their C-terminal targeting domains must be normally folded. We also developed a novel protein FRET substrate (MV11) for the endopeptidase activity of lysostaphin by introducing a pentaglycine target sequence into the N-terminal 57 residues of colicin E9. Mass spectrometer analysis demonstrated that cleavage of the pentaglycine target sequence in MV11 occurred between position 2 and 3.

Lysostaphin producing strains are resistant to killing by lysostaphin due to the incorporation of serine residues at position 3 and 5 of the pentaglycine linker in their cell walls. The MV11 substrate was engineered to introduce a serine residue in turn at all five positions of the pentaglycine target site. Cleavage of the serine containing substrates was monitored by SDS-PAGE and revealed that only a serine residue at position 3 completely inhibited cleavage. This suggests that the incorporation of a serine residue at position 3 in the pentaglycine linker in the cell wall will be sufficient to cause resistance to lysostaphin in *S.aureus* cells.