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BACTERIOCINS are ribosomally-synthesized antimicrobial peptides produced by bacteria. Most LAB bacteriocins are pore formers but some are also active as cell wall inhibitors by targeting cell wall precursors.

Lcn972 is a bacteriocin that inhibits cell wall biosynthesis at the division septum by binding to lipid II. It is active exclusively against other lactococci and lacks any post-translational modifications. These features make Lcn972 an attractive molecule as template for developing new antibiotics as it may bear a new lipid II binding domain. Unfortunately Lcn972 unfolds irreversible at room temperature preventing its use to map the interactions with lipid II.

**Structure of Lcn972**

Lcn972 is rather compact and folds as a β-sandwich comprising two three-stranded antiparallel β-sheets (PDB:2LGN).

Glycerol preserves the folded active form of Lcn972.

**Lcn972 variants**

Cys codons were introduced by inverse PCR on the nisin-inducible lcn972 expressing plasmid pBL54 and the mutated plasmids were introduced into L. lactis NZ9000. Inhibitory activity was retained by the Lcn972 variants N30CA59C and S15CA26C.

Unfolding of Lcn972 and its variants was monitored by tryptophan fluorescence (Ex. 280 nm) at 37 °C. Unfolding was partially prevented in N30CA59C and delayed in S15CA26C. Presence of DTT (20 mM) restored wildtype values.

**CONCLUSIONS**

- The structure of Lcn972 is unique among LAB bacteriocins and other lipid II binders.
- Covalent linking of both halves of the β-sandwich slows down unfolding but impairs activity.

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