IMPROVING THERMAL STABILITY OF THE METASTABLE BACTERIOCIN LCN972

Andreia P. Cepeda[1], Silvia Portilla[2], Ana Rodríguez[2], Teresa Catarino[1], David L. Turner[1] and Beatriz Martínez[2]*
[1]Cell Physiology & NMR group, ITQB-UNL, PORTUGAL: [2]DairySafe group, IPLA-CSIC, SPAIN.
* contact: bmf1@ipla.csic.es

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.

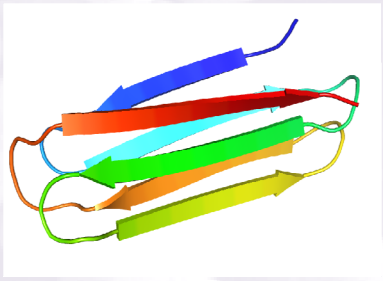
Lcn972 is a bacteriocin that inhibits cell wall biosynthesis at the division septum by binding to lipid II. It is active exclusively against other *lactococci* and lacks any post-translational modifications. These features make Lcn972 an attractive molecule as template for developing new antibiotics 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.

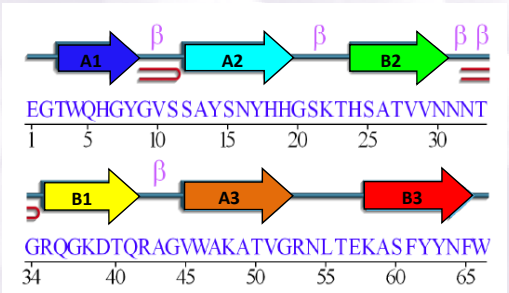
GOALS

- To solve the 3D structure of Lcn972 by NMR
- To introduce disulfide bridges to prevent Lcn972 unfolding

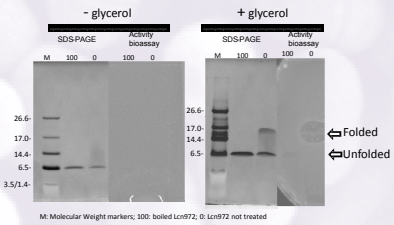
Structure of Lcn972

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





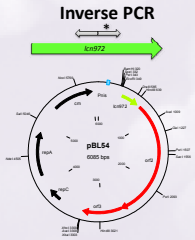
Glycerol preserves the folded active form of Lcn972



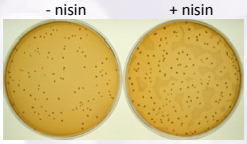
Lcn972 variants

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.

Inverse PCR



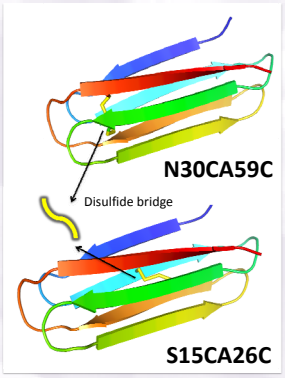
Inhibitory activity test



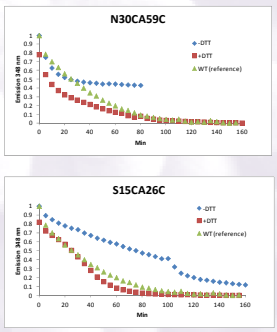
Peptide	Specific activity (AU/μg)
WT	95.5
V29CS60C	0
N30CA59C	5
S15CA26C	50

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.

Lcn972 variants



Peptide unfolding



Peptide	Half-life at 37 °C (min)	
	- DTT	+ DTT
WT	22.4±1.5	25.3±3.4
N30CA59C	ND	15.7±9.2
S15CA26C	45.5±6.5	30±7.3

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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Grant BIO2010-17414 (Ministerio de Economía y Competitividad -Spain) and PEstOE/EQB/LA0004/2011,
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