

SESSION I: SPEAKER ABSTRACTS

Mode of Action Studies of the S-linked Glycocin

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Sublancin is a 37-amino acid antimicrobial peptide belonging to the glycocin family of natural products. It undergoes several post-translational modifications, with two disulfide bonds connecting the two helices and a glucose installed on a cysteine residue. We reported the reconstitution of the biosynthetic pathway to this natural product in *Escherichia coli*. This technology enabled the evaluation of the structure activity relationships of the solvent exposed residues in the helices and the bioactivity studies of the resulting mutants show that two residues in helix B, Asn31 and Arg33 as well as the sugar moiety attached to the peptide are critical for its bioactivity. We additionally examined the mode of action of sublancin by monitoring macromolecular synthesis. Sublancin negatively affected DNA replication, transcription, and translation without affecting cell wall biosynthesis. In addition, we confirmed that the presence of the PTS sugar glucose in the medium negatively impacted antimicrobial activity of sublancin. Additionally, sublancin analogues carrying different sugars retained their antimicrobial activity regardless of which sugar was attached to the peptide or the carbon source used. These data suggest a novel mechanism upstream of transcription and translation and are consistent with previous studies suggesting that the glucose uptake system is involved. Additionally, overexpression of any of the pts genes in sensitive *Bacillus* strains increases their sensitivity. Collectively, the results indicate sublancin leads to a yet unknown gain of function of glucose PTS. Investigations are in progress to tease out the factors affecting sublancin sensitivity and elucidate the target.

