

## MS08-P03 | TRAF IS AN ESSENTIAL FACTOR OF THE PIP501 TYPE IV SECRETION SYSTEM (T4SS) AND EXHIBITS STRUCTURAL HOMOLOGY WITH THE T7SS PROTEIN ESSB OF STAPHYLOCOCCUS AUREUS.

Stallinger, Amrutha (University of Graz, Graz, AUT); Kohler, Verena (University of Graz, Graz, AUT); Lammer, Anna (University of Graz, Graz, AUT); Berger, Tamara (University of Graz, Graz, AUT); Probst, Ines (University Medical Center Freiburg, Freiburg, GER); Grohmann, Elisabeth (Beuth University of Applied Sciences, Berlin, GER); Keller, Walter (University of Graz, Graz, AUT)

The rapid spread of antibiotic resistances and the occurrence of multi-resistant strains among pathogenic bacteria, in particular those causing nosocomial infections, is one of the most pressing problems of our health system. Bacterial conjugation is the most prevalent route of DNA transfer between bacteria and mediates the rapid spread of bacterial resistances within bacterial communities. Type IV secretion systems (T4SS) are responsible for the efficient transport of nicked, single-stranded plasmid DNA across the cell walls of the donor as well as the recipient cell <sup>1</sup>. The T4SS from the antibiotic resistance plasmid pIP501, occurring in *Enterococci* and related Gram-positive bacteria, is encoded within a single operon comprised of 15 putative transfer factors. The 6<sup>th</sup> open reading frame encodes TraF, a bitopic trans-membrane protein of 52.8 kDa. Here we present the crystal structure of the N-terminal, cytosolic domain determined at 1.4 Å and show that TraF<sub>N</sub> exhibits a surprising structural homology with the EssB proteins of *Staphylococcus aureus* and *Geobacillus thermodenitrificans*. The solution structure of both the N- and the C-terminal domains were determined by SAXS methods. Western blot analysis revealed that TraF is part of the isolated T4SS complex. Interaction studies using the bacterial-two-hybrid system (BACTH) showed that TraF and its domains interact with the VirB8-like protein TraM, which was proposed to be an integral part of the functional T4SS <sup>2,3</sup>. Generating knock-out mutants we proved that TraF is an essential component of the transfer machinery, as the transfer rates were basically abolished when the TraF gene was missing.