

Structural and functional characterization of a DNA binding protein of pIP501– a broad-host-range plasmid

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The spread of resistances against antibiotics in bacteria is a serious global problem. In order to prevent the transfer of resistances it is crucial to understand the involved processes. Conjugative DNA transfer is the most important means to transfer antibiotic resistance genes among bacteria. It is present in Gram- positive (G⁺) and in Gram- negative (G⁻) bacteria.

I am working on a Type IV Secretion System (T4SS) encoded on the broad-host-range plasmid pIP501 from *Enterococcus faecalis*. It can spread among different types of bacterial hosts and hence plays an important role in the propagation of multi drug resistant germs. *Enterococci* are abundant among humans and animals, which intensifies the problem.^[1] To date most of the structural information stems from G⁻ T4SS.^[2] Deciphering the mechanisms involved and solving the structure of the pore forming complex (PFC) would be of great help in the war against multidrug resistant bacteria.

Alongside the structural elucidation of the PFC, I am working on a DNA binding protein, namely TraM, which is a putative member of the PFC. The investigation of TraM includes biophysical, biochemical and structural characterization.

We are on the way to determine the residues of TraM, which are involved in DNA binding. We designed two different N-terminal constructs varying in length, TraM94 and TraM167. The structure of TraM94 was recently solved in our group. In contrast to the monomeric TraM94 TraM167 is a trimer in solution like the C-terminal domain of TraM, whose structure was solved in our group already some years ago.^[3]

- [1] V. Kohler, A. Vaishampayan, and E. Grohmann, “Broad-host-range Inc18 plasmids: Occurrence, spread and transfer mechanisms,” *Plasmid*, vol. 99, pp. 11–21, Sep. 2018.
- [2] J. M. Chung *et al.*, “Structure of the helicobacter pylori cag type IV secretion system,” *Elife*, vol. 8, pp. 1–15, 2019.
- [3] N. Goessweiner-Mohr *et al.*, “The 2.5 Å Structure of the Enterococcus Conjugation Protein TraM resembles VirB8 Type IV Secretion Proteins,” *J. Biol. Chem.*, vol. 288, no. 3, pp. 2018–2028, Jan. 2013.

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