Functional characterization of ClpP mutations conferring resistance to ADEP antibiotics in Firmicutes

Malik IT[a], Pereira R[a][b], Vielberg MT[c], Mayer C[a], Groll M[c], Brötz-Oesterhelt H[a]*

[a] Interfaculty Institute of Microbiology and Infection Medicine, Dept. of Microbial Bioactive Compounds, University of Tuebingen, Auf der Morgenstelle 28, 72076 Tuebingen, Germany
[b] Laboratory of Antibiotics, Biochemistry, Education and Molecular modeling, Department of Molecular and Cell Biology, Federal Fluminense University, Outeiro São João Batista, Centro, Niterói, Rio de Janeiro, 24210130 Brazil
[c] Center for Integrated Protein Science at the Department of Chemistry, Technical University Munich, Lichtenbergstrasse 4, 85748 Garching, Germany

ADEP (acyldepsipeptide) is an exploratory antibiotic with a novel mechanism of action. ClpP, the proteolytic core of the caseinolyic protease, is deregulated towards unrestrained proteolysis. Here, we report on the mechanism of ADEP resistance in Firmicutes, as this bacterial phylum contains important pathogens to be potentially targeted by future ADEP therapy. For *Staphylococcus aureus*, *Bacillus subtilis*, enterococci and streptococci, spontaneous ADEP-resistant mutants were selected *in vitro* at a rate of 10^{-6}. All isolates contained mutations in ClpP. Characterised mutated *S. aureus* ClpP proteins were out-of-function and provide insight into the operation mode of ClpP. For molecular insights crystal structures of *S. aureus* ClpP bound to ADEP4 were determined. Well-resolved N-terminal domains in the *apo* structure allow to follow the pore-gating mechanism. The mutant compilation presented indicates residues relevant for ClpP function and suggests that ADEP-resistance will occur at a lower rate during the infection process.