Project 08 : High-throughput characterization of *Pseudomonas aeruginosa* genes of unknown function (Dr. Filip Kovacic and Prof. Karl-Erich Jaeger)

Background: *Pseudomonas aeruginosa* causes severe hospital-associated infections, especially in immunocompromised persons, which are complicated to treat due to the increasing antibiotic resistance and high cytotoxicity of this bacterium. However, nearly half of the genes of *P. aeruginosa* were never experimentally characterized. This fact is surprising if one knows that this bacterium:

(i) belongs, according to the World Health Organization, to the most critical group of pathogens for research and development of novel antibiotics,

(ii) represents a model-pathogen for studying the development of biofilms which are certainly the most significant antibiotic resistance determinants,

(iii) comprises all currently known bacterial antibiotic resistance mechanisms.

Own previous work: The comprehensive sequence homology and 3D structure prediction analyses of 2200 *P. aeruginosa* proteins encoded by genes of unknown function revealed putative virulence and antibiotic-resistance determinants. In a high-throughput approach, we have synthesized by PCR all genes of unknown function and cloned most of them into a newly developed pGUF expression plasmid suitable for heterologous expression in *E. coli* and homologous expression in *P. aeruginosa*. In the first high-throughput experiments, enzymatic functions of several proteins encoded by genes of unknown function were identified.

Aim of the project: We aim to experimentally characterize biochemical functions of proteins encoded by genes of unknown function to identify novel putative resistance and virulence factors of *P. aeruginosa*.

Work program: In a functional screening of proteins of unknown function, we will express and identify novel toxins and antibiotic-resistance proteins, folowed by verification of their functions with purified proteins. Using a comprehensive *P. aeruginosa* transposon mutant strain collection we will identify antibiotic-resistance and virulence phenotypes associated with newly identified proteins of unknown function.