Investigating bacterial stress responses with chemical genetics

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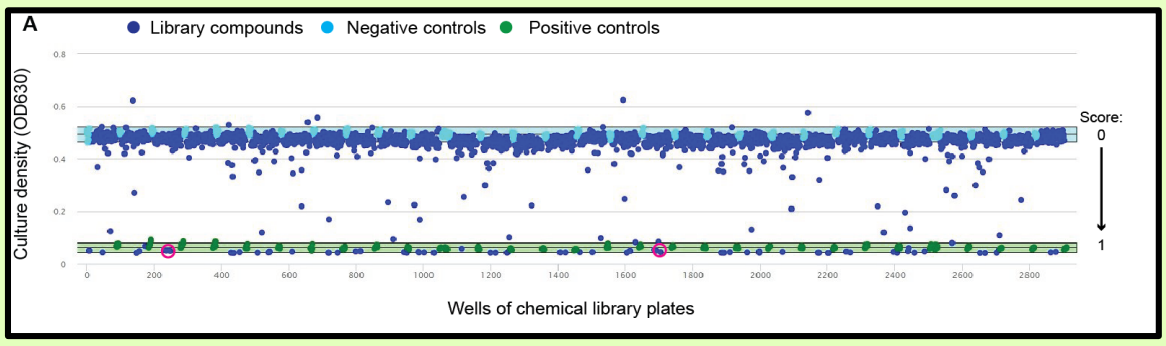
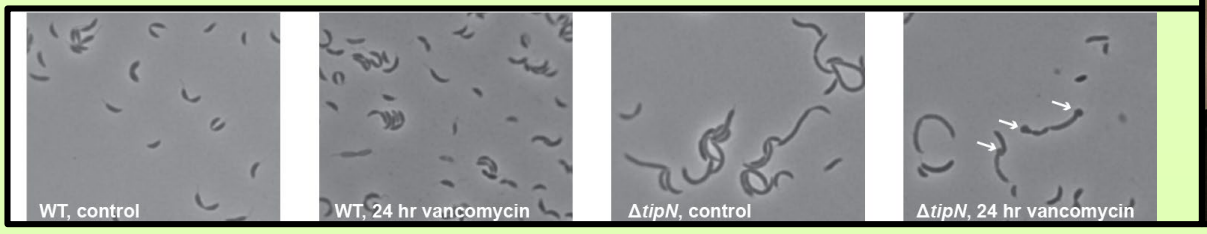
Main Research Areas

Bacteria in their natural environment can encounter a huge range of stress factors, from physical (temperature, mechanical stress), chemical (oxidative stress, exposure to toxic chemicals) and biological (the human immune system, bacteriophages, antibiotics secreted by other micro-organisms) sources. Success and proliferation in their environment requires a wide repertoire of mechanisms to detect and respond to these stress factors. We are particularly interested in the responses of the alpha- and gamma-proteobacteria to antibiotic stress, as these classes of bacteria contain many clinically and agriculturally important pathogens. We use *Caulobacter crescentus* and *Pseudomonas aeruginosa* as model organisms and study the mechanisms of (a) the cell envelope stress response, (b) DNA damage response, including toxin-antitoxin systems, and (c) bacterial defence against bacteriophages, in their response to and/or protection against these stress factors. A key method is the use of chemical-genetic high-throughput screening to detect novel chemical or antibiotic compounds with selective toxicity for particular mutant strains of interest, but we also employ classical bacterial genetics and genomics as well as biochemical assays and microscopy to pinpoint the molecular mechanisms of gene-drug interactions that we discover.

If you are interested in any of our projects, please contact: clarek@bmb.sdu.dk





Projects	Description
Role of a *Caulobacter* two component system, ChvIG, in cell envelope stress	Unusually for a Gram-negative bacterium, *Caulobacter crescentus* is relatively sensitive to vancomycin. A suppressor screen for mutations that could confer vancomycin resistance led to a TonB-dependent receptor, ChvT. This gene is regulated by a two-component system named ChvIG, which is highly conserved between *Caulobacter* and the symbiotic and pathogenic alpha-proteobacteria, and usually involved in sensing that the bacteria are associated with a host cell. In *Caulobacter*, which is free-living, it appears to have been re-purposed for sensing cell envelope stress. We are currently working on defining the targets of ChvIG.
An unusual three-component toxin-antitoxin system, HigBAC, in *Caulobacter*	We previously characterised a toxin-antitoxin system of *Caulobacter crescentus*, HigBA, which was involved in the SOS (DNA damage) response of this bacterium and found that HigB toxin activity contributed to cell death on exposure to DNA damaging antibiotics. However, *higBA* deletion mutants had some unexpected phenotypes, so we investigated whether the next gene downstream of *higBA*, an unannotated putative transcription factor, was the cause. We found that this gene, now named *higC*, was in the HigBA operon and that it also influenced survival with DNA damaging antibiotics, but that it was probably capable of regulating other genes as well as the *higBA(C)* promoter. We are currently investigating how it regulates its own expression and what are its other targets in the genome.
Bacteriophage defence mechanisms of *Pseudomonas aeruginosa*	Bacteriophages are (re)gaining the interest of researchers as a possible alternative to antibiotics, in the event that multidrug resistance becomes uncontrollably prevalent. However, bacteria have always been exposed to bacteriophages in their natural environments and have evolved mechanisms to defend against them. It is essential to know what bacteriophage defence mechanisms a given pathogen uses if “phage therapy” is to become a realistic proposition. However, knowledge on this subject is lacking. We have used transposon insertion-deep sequencing to identify possible phage resistance genes in *Pseudomonas aeruginosa*, and discovered that structural genes of the type VI secretion system may be involved in bacteriophage defence. Characterization of the molecular mechanism behind this phenomenon is a future goal of the group.