EuroMicropH
Acidic Fridays

3rd Acidic Friday 2021-02-19 16:00 CET

Open discussion platform of the COST action EuroMicropH. This discussion series is intended to stimulate an exchange on the different aspects of how microorganisms react to low pH conditions and why people are interested to investigate this subject.

Please register here for the upcoming meeting. To access the meeting please follow the Zoom link beneath.

Join Zoom-Meeting

https://tuwien.zoom.us/j/99837640693?pwd=dnY1RDZaeUVyZjRzKzZaSlpEMjdNZz09

Meeting-ID: 998 3764 0693 Passwort: bcS94kj5

Agenda

Chairs

Nuno PEREIRA MIRA, Instituto Superior Técnico (IST) , Portugal

Zeynep ÇETECIOĞLU-GÜROL, KTH Royal Institute of Technology, Sweden

Programm

16:00 Welcome

16:05 Gerd SEIBOLD, DTU Bioengineering, Denmark

Sensor-based visualization of the cytoplasmic pH in agar-plate-based colony array for high-throughput studies on pH-homeostasis in *Escherichia coli*

16:25 Binu KUNDUKAD, SCELSE, Nanyang Technological University, Singapore

Weak acids as an alternative anti-microbial therapy

Questions and Discussion

Do you want to contribute to upcoming Acidic Fridays? Please register a talk here or contact Matthias Steiger Matthias.steiger@tuwien.ac.at (Leader WG 6).

Abstracts

Gerd Michael Seibold, DTU Bioengineering, Technical University Denmark

Sensor-based visualization of the cytoplasmic pH in agar-plate-based colony array for high-throughput studies on pH-homeostasis in *Escherichia coli*

Cytoplasmic pH is a physiological parameter which underlies relatively tight regulation in bacterial cells. This active process termed pH-homeostasis is achieved via the interplay of diverse mechanism such as buffering, adaptations of membrane structure, active ion transport, metabolic consumption of acids and bases and involves a variety of interconnected regulatory processes for the coordination. Many of the processes and regulators underlying pH-homeostasis have been identified in genetic studies using strain libraries and phenotypic screening for non-growth at low or high pH-values. Genetically encoded pH sensor proteins like ratiometric pHluorin have been used for real-time monitoring of pH-homeostasis processes within living bacterial cells.

In this communication we show imaging of the internal pH-dependent, ratiometric fluorescence signals of the sensor protein pH-Cherry in *E. coli* MG1655 colonies cultivated on agar plates and used this approach to distinguish between strains with altered pH-homeostasis. Combining this imaging technology with library generation via transposon mutagenesis, as well as robot-assisted colony picking and replica plating allowed us to identify six mutant strains with altered internal-pH values from a small library of 500 mutants at non-selective growth conditions. Characterization of the mutant strains in liquid cultures at different pH-values confirmed their altered pH-homeostasis profiles when compared to the parental strain. Identification of the transposon insertion sites revealed that indeed six different genes were inactivated in the six identified mutants and all of these genes are to be to some extent involved in regulation of or pH-homeostasis itself. These proof of concept experiments show that sensor-based analyses of internal pH of colonies on agar plates is a sensitive approach for the fast identification of interesting genes involved in pH-homeostasis in E. coli and can be applied for high-throughput analyses of pH-homeostasis in further strains and bacteria.

Binu Kundukad, SCELSE, Nanyang Technological University, Singapore

Weak acids as an alternative anti-microbial therapy.

Selective permeability of a biofilm matrix to some drugs has resulted in the development of drug tolerant bacteria. We studied the efficacy of weak organic acid drugs, such as N-acetyl-L-cysteine (NAC), on the eradication of biofilms formed by *P. aeruginosa* and the commonality of this drug with other acids such as acetic acid and formic acid. We showed that monoprotic acids at pH < pKa can penetrate the matrix and eventually kill 100% of the bacteria embedded in the biofilm. The efficacy of a monoprotic weak acid (NAC) and triprotic weak acid (citric acid) on biofilms formed by different bacterial strains as well as an antibiotic resistant biofilm were also investigated. We found that weak acids are effective in eradicating persister cells in biofilms which are tolerant to other conventional methods of biofilm eradication. The study also includes the response of the biofilm matrix to the treatment of weak acids.