EuroMicropH

Acidic Fridays

21st Acidic Friday 19.05.2023 15:00 CEST

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 session, please follow the zoom link below.

19.05.2023 15:00 CEST

Join the Zoom-Meeting

https://tuwien.zoom.us/j/66178441262?pwd=NWhYWUlEeENua2FDSTJVeXhxaVp3Zz09

Meeting-ID: 661 7844 1262

Password: Jn9m40zL

Agenda

Chairs: **Aleksandra Djukić-Vuković**, University of Belgrad, Serbia; **Ricardo Santos**, IST, Portugal

Programm

15:00 Welcome

15:05 Rebecca Hall, University of Kent, UK

How the fungal pathogen *Candida albicans* adapts to acidic environments

15:25 Styliani Roufou, University of Malta, Malta

Evaluation of microbial resistance under stress pH and temperature levels of eGFP-labelled *Escherichia coli* strains

15:45 Quinten Goessaert, KU Lueven, Belgium

In vivo functional characterization of GadC alleles that cause hyper antibiotic tolerance in *E. coli*

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Abstracts

How the fungal pathogen *Candida albicans* adapts to acidic environments?

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Candida albicans is able to proliferate in environments that vary dramatically in ambient pH, a trait required for colonising niches such as the stomach, vaginal mucosal and the GI tract. Adaptation of *C. albicans* to acidic environments induces active cell wall remodelling, resulting in the exposure of the underlying beta-glucan and chitin. This modification of the cell wall structure results in alteration of the host-pathogen interaction, which is largely mediated via increased Dectin-1 dependent recognition of the unmasked beta-glucan. Here we show that adaptation of *C. albicans* to acidic environments is a highly dynamic process. Initial exposure of *C. albicans* to acidic conditions, results in the structural rearrangement of the cell wall, resulting in unmasking of both beta-glucan and chitin with 2 hours. However, over time, *C. albicans* is able to re-mask its cell wall concealing the highly proinflammatory beta-glucan. Global transcriptional approaches identified a core set of 22 genes that are differentially regulated by pH independently of time, and subsets of genes that are differentially regulated by both pH and time. This global transcriptional analysis has elucidated the molecular mechanism by which *C. albicans* re-masks its cell wall during recovery from the initial acid shock. Therefore, within the host *C. albicans* is constantly remodelling its cell wall which will have a significant impact on the host-pathogen interaction at various stages of the infection.

Assessment of microbial resistance under different pH and temperatures based on eGFP-labelled strains

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The food industry uses hydrochloric acid to process products such as hydrolyzed vegetable proteins. This treatment lowers the initial pH, which prevents microbial growth, while its efficacy is strongly related to temperature. The presence of pH and temperature stress conditions results in the expression of certain genes, which protect the organism. The impact of stress on microbes should be assessed to understand the mechanism of this response. In this study, the effect of temperature and pH on the

growth of eGFP-tagged *Escherichia coli* BW25113 and its isogenic deletion mutants Δ*rpoS, ΔdnaK* and Δ*gadB* strains were assessed in Miller's Lysogeny Broth. Hydrochloric acid was used to adjust the pH. Four different temperatures and pH levels, ranging from 27 to 42°C and 4.5 to 7, respectively, were studied in a microplate fluorospectrometer. The specific growth rate was estimated using the generation time. *Escherichia coli* stress tolerance under different *pH* and *temperature* scenarios was mathematically modelled through different mathematical expressions, i.e., polynomial-type models. At temperatures above 35°C, for all pH conditions, *E. coli* Δ*dnaK*-eGFP strain had the lowest growth rates of the other tested strains, below 1.07 1/h. While at 27°C, it grew faster than the other strains, indicating its importance in heat shock resistance. The reverse phenomenon occurred under the same conditions in the *E. coli* Δ*rpoS*-eGFP strain. Its growth rate increased by 0.84 to 1.47 1/h when the temperature and pH rose; this trend demonstrates the importance of the *rpoS* gene in acid and low-temperature resistance. Additionally, *E. coli* Δ*gadB*-eGFP grew faster than *E. coli* BW25113-eGFP at 32°C and pH 4.5, likely due to the involvement of the homologous GadA protein in the absence of GadB. Furthermore, the response surface modelling approach gave saddle points for all strains. Specifically, *E. coli* BW25113-eGFP strain had a saddle point at 19.83°C and a pH of 5.96. This study highlighted the need to facilitate the development of new food safety approaches for elucidating the action mechanism of microbial pathogens on pH. This is the first study to highlight the fundamental importance of genes involved in the GAD system of *Escherichia coli* under acidic and temperature stress conditions.

In vivo **functional characterization of GadC alleles that cause hyper antibiotic tolerance in *E. coli***

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Antibiotic persistence is the presence of a subpopulation within an isogenic population that tolerates an otherwise lethal antibiotic treatment. Through evolution experiments with E. coli we identified mutations in the glutamate/GABA antiporter GadC involved in acid resistance which result in extreme levels of antibiotic-tolerant persister cells. The goal of my STSM was to characterize different acid resistance phenotypes in antibiotic tolerant E. coli expressing different GadC alleles discovered by dr. Bram Van den Bergh (VIB-KU Leuven, Belgium). To this end, I quantified GABA levels with the GABase assay, determined acid resistance (AR) and performed HPLC assays were applied, which are consolidated techniques in the lab of prof. Daniela De Biase (Sapienza, Italy, the host of the STSM).