BOOK OF ABSTRACTS

HDID V

How dead is dead?

5th conference on exploring the edge of bacterial life

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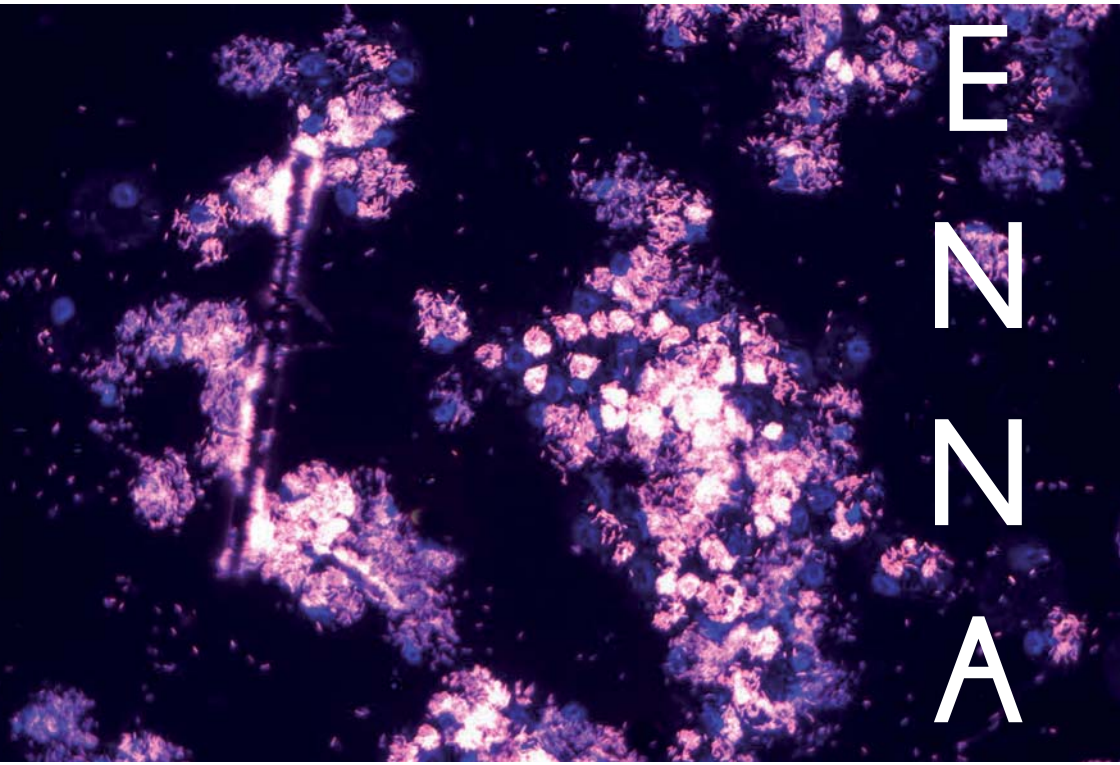

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September 6-8, 2017

Universitätszahnklinik, Sensengasse 2a, 1090 Vienna

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ORAL PRESENTATIONS

WELCOME LECTURE

Invited talk

Life on the hold: a recipe for disaster

Wolf-Dietrich Hardt
ETH Zürich, Institute of Microbiology, Zürich, Switzerland

Bacteria have evolved numerous strategies to survive periods of nutrient deprivation and the exposure to noxious substances. I will review emerging principles and use our work on *Salmonella* Typhimurium to illustrate some grave consequences of persistence. *Salmonella* Typhimurium is a Gram-negative pathogen and a common cause of diarrhea. This disease cannot be treated with antibiotics. This is attributable at least in part to the pathogen's ability to form slow-growing populations within the host's dendritic cells. Importantly this slow-growing subpopulation is recalcitrant to antibiotic therapy. Moreover, these slow growing pathogen populations can cause relapses after the end of the therapy. This has some striking consequences for pathogen evolution, which will be discussed.

OPENING LECTURE 1

Invited talk

What have we learned since the first How-dead-is-dead conference?

Hans-Curt Flemming[1,2] and Jost Wingender[1]

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[2]Singapore Centre for Environmental Life Sciences Engineering (SCELSE), 60 Nanyang Drive, Singapore 637551

The question posed at the first HDID conference in Bochum, Germany, in 2009 was straightforward: when are bacteria really dead? The conference title challenged the common view that absence of culturability is equivalent to cell death. Research had shown that selected health-relevant bacterial species, which normally could be cultivated, refused to grow as a response to stress such as starvation, oxygen concentration, exposure to toxic metals, desiccation etc., but still showed life signs such as membrane integrity and membrane potential, respiration, protein synthesis, transcriptional activity or active efflux systems. The most important feature was that some of them could be resuscitated, and, in case of pathogens, could regain virulence. They were termed "viable but non-culturable" (VBNC). In the meantime, the relevance of the VBNC condition has been acknowledged in many fields, e.g., medicine, food production or drinking water safety. It is obvious that cell death can only be confirmed by suitable culture-independent methods (as desired, e.g., in disinfection procedures). The most common criteria to date comprise the structural and functional loss of membrane integrity or DNA. Resuscitation on the other hand remains the most compelling parameter for assessing relevance to human health as only very few cases have been reported of cells being virulent in their VBNC state. Still there are many open questions about which conditions actually induce this state and, even more important, under which real, non-laboratory conditions, resuscitation has to be expected. Until now, most studies are descriptive rather than elucidating mechanisms. Furthermore, it remains still unclear how to rate health risks associated in detection of VBNC organisms in a drinking water system, what to do about them and how to prevent them. Practitioners, therefore, have a very sober look at our research – we still owe them some answers.

OPENING LECTURE 2

Invited talk

Identical or Different: Viable but Non Culturable, Persister, Strategic, and/or Dormant?

Rita R. Colwell[1,2], Salvador Almagro-Moreno[3,4]

[1]University of Maryland College Park; [2]Johns Hopkins University; 3Bloomberg School of Public Health [4]Burnett School of Biomedical Sciences, University of Central Florida

Unlike Gram-positive bacteria, Gram-negative bacterial cells historically had been considered vulnerable to adverse environmental conditions, with exceptions being those adapted to extreme environments.In the early 1980's, demonstration of the survivability of these bacteria after exposure to external stressors, even though no longer able to be cultured, was successful by employing both direct and indirect methods.Initially, controversial, this phenomenon has been demonstrated in many bacterial species and publications now number in the thousands that deal with viable but non culturable bacteria.In the clinical laboratory, the persistence of cells of an otherwise antibiotic sensitive bacterial culture after antibiotic treatment raised new questions concerning viability and its role in public health.Novel developments in the study of the molecular mechanisms involved in the viable but non culturable state (VBNC), have contributed to greater understanding of the genetic underpinnings regulating these phenomena.Most recent are results of studies showing phenotypic differences among cells of pure cultures, overall, offering more questions than answers but a very intriguing phenomenon. Here, we will provide an overview and a synthesis of the conditions and mechanisms that differentiate cells in the VBNC state from those in other persistent and/or dormant

Effect of temperature, starvation and predation in the survival of Vibrio harveyi populations

Maite Orruño[1,2], Claudia Parada[1], Elixabet Ogayar[1], Vladimir R. Kaberdin[1-3], Inés Arana[1,2]

[1]Department of Immunology, Microbiology and Parasitology. University of the Basque Country (UPV/EHU), Spain; [2]Research Centre for Experimental Marine Biology and Biotechnology (PiE-UPV/EHU), Spain; [3]IKERBASQUE, Basque Foundation for Science, Spain

The survival of *Vibrio* spp. in natural environments is conditioned by biotic and abiotic factors. Microbiologists have traditionally focused their attention in the study of abiotic factors inducting of the viable but nonculturable state (VBNC), phenotype described as a strategy to persist under adverse conditions. Predation by protozoa has been identified as a major mortality and selection factor for bacteria populations in aquatic environments, affecting their abundance, composition and activity. The aim of this work was to study the importance of predation and of induction of VBNC in the survival of vibrios at different temperatures.

Survival assays were carried out maintaining the *V. harveyi* ATTC 14126 strain in absence of seawater microbiota and a *gfp*-tagged *V. harveyi* strain (distinguishable from indigenous seawater bacteria) in untreated seawater. Samples were incubated at 4°C, 12°C, 20°C and 30°C during 21 days. Along the experiments, total and active *V. harveyi*, as well as total ciliated and flagellated protozoa, were enumerated via epifluorescence microscopy, and culturable bacteria were determined on marine agar. In absence of predators, starvation induced the entry into the VBNC state, especially at low temperature (culturable fraction decreased 3.5-log, while viable population remained unchanged). In presence of indigenous microbiota, an initial period of stability was followed by a pronounced decline of total and culturable *V. harveyi*, together with the increase of protozoa. The length of this process was variable depending on the temperature. Finally, when *V. harveyi* population declined below a threshold level, the predatory pressure was relaxed and the surviving cells adopted the VBNC state.

These results suggest that predation is a temperature-regulated factor controlling *V. harveyi* population density in natural environments, while induction of VBNC state below a threshold density could guarantee the persistence of survivor bacteria that escape predation.

Bacterial necrotrophic growth on dead-biomass in drinking water

Ioanna Chatzigiannidou[1], Ruben Props[1,2], Nico Boon[1]

[1]Center for Microbial Ecology and Technology(CMET) - Ghent University; [2]Belgian Nuclear Research Centre (SCK•CEN)

Water sources nowadays are disinfected either chemically (e.g. chlorination) or physically (e.g. heat and UV). Such methods result in the reduction of bacterial loads by orders of magnitude. However, they cannot ensure that all microorganisms are killed, and concurrently the dead bacterial biomass may act as a carbon and nutrient source for the surviving bacteria. The capacity of bacterial strains to grow on dead-bacterial cells has been described before as necrotrophy or necrotrophic growth. In this study, we investigated the effects of necrotrophy on the specific selection of bacteria in drinking water. More particularly, we studied how the addition of pre-killed bacterial biomass influences the bacterial concentrations and the bacterial community structure of bottled water. Heat-killed or fumigated-killed cells of a Gram+ (*Lactobacillus brevis*) and a Gram- (*Escherichia coli*) representative were added in bottled water. The cell concentrations of the samples were monitored over seven days by flow cytometry and the community composition was assessed at the end of the experiment by 16S rRNA gene amplicon sequencing. We observed that necrotrophic growth took place with the bacterial community growing from an initial concentration of 10^4 cells/mL to a final concentration 10^7-10^8 cells/mL. The community structure differed between the dead-cell treated and the non-treated samples and certain bacterial genera have been significantly more enriched during necrotrophic growth. Finally a general decrease in community diversity (phenotypic and taxonomic) was observed for all treated samples compared to non-treated water.
We showed that different indigenous bacterial taxa of natural aquatic microbial communities can grow on dead-biomass. The increased cell numbers in combination with the specificity of the taxa that grow necrotrophically can alter heavily the microbial community.

Detection of VBNC Vibrio cholerae by rt-qPCR after transcriptome analysis using RNA-seq

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The viable but nonculturable (VBNC) state is an adopted survival strategy by bacteria when they are exposed to adverse environmental conditions. Several methods have been developed to detect the presence of VBNC bacteria, such as checking membrane integrity, enzymatic activity and respiration rate among others. Nevertheless, detection methods for VBNC bacteria present a controversial debate related with what viability assays actually mean in terms of the status of most microorganisms.

For this reason, it is thus necessary to develop methods in order to definitively detect and quantify VBNC bacteria in a simple one-step method.

In this study we induced the VBNC state of *Vibrio cholerae* through two conditions. First by low temperature and oligotrophic media (Artificial Sea Water) and the same conditions but and inductor of biofilm (*c-di-GMP*) was added.

Induction of the VBNC state was followed by conventional cultivation-based techniques and sodium pyruvate supplemented media to discard injured bacteria. Bacteria viability was checked by membrane integrity using the LIVE/DEAD BacLight bacterial viability kit and CTC staining with 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) to detect respiratory activity.

Three conditions were analyzed by Ion-Proton RNA-seq technology with three biological replica. The Exploratory analysis and differential expression for RNA-seq data was done by NOISeq. Fold-change of 2657 genes was observed and the resulting simulated fold-change between the average expression of the three conditions after normalizing data varied from 1.3 to 1100 with a median value of 42. The PhoB gene related with the phosphate responsive regulator was selected and primers for rt-qPCR were design to detect bacteria in five metabolic states (exponential growth, late stationary phase, 10 days induce. 6 moths induced bacteria and dead bacteria by chlorine). Results show the feasibility of use the PhoB gene to detect VBNC *V. cholerae* with a 95% confidence in contrast with 75% of working with non analyzed gene targets.

SESSION 2: RESUSCITATION

Invited talk

Resuscitation from the VBNC State

James D. Oliver[1,2]

[1]Department of Biological Sciences, University of North Carolina, Charlotte; [2]Adjunct Professor, Duke University

This review will begin with a brief summary of the VBNC state in *Vibrio vulnificus*, then concentrate on resuscitation from this dormancy state. This will include a summary of various agents and environmental triggers shown to induce resuscitation, ranging from temperature changes to "resuscitation promoting factors". The role of quorum sensing, possible molecular mechanisms involved, and our recent findings on gene expression during resuscitation will be described. Finally, implications for global warming will be discussed.

Resuscitation of viable but non-culturable (VBNC) Vibrio vulnificus results in dramatic changes in gene expression

Tiffany C. Williams[1], Seongwon Kim[2], Gary Vora[2] and James D. Oliver[1,3]

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The viable but non-culturable (VBNC) state is a well-recognized form of bacterial dormancy, in which exposure to stressful environmental conditions cause populations to cease growth and to escape detection using culture-based methods. Over 100 bacterial species have been reported to exist in the VBNC state including the seafood-borne opportunistic human pathogen, *Vibrio vulnificus*. This dormancy mechanism is presumed to be a common survival mechanism employed by bacteria, thus a better understanding of the molecular processes governing VBNC cell formation and resuscitation is imperative in order to properly assess the risk these non-culturable populations pose to human health. The objective of the current study was to comprehensively investigate the transcriptional mechanisms facilitating VBNC dormancy mechanisms using temporal transcriptomics. To do this, *Vibrio vulnificus* was incubated in artificial seawater microcosms (in triplicate), placed at 4°C to induce the VBNC state and monitored for culturability over time. Once the entire population was unculturable resuscitation was induced by incubation at 22°C. Samples were harvested from log phase cells and on days 0 (pre-VBNC), 2 (early VBNC), 14 (late VBNC), and after resuscitation. Samples were subjected to RNA extraction, rRNA depletion, and Illumina transcriptome sequencing. Pairwise comparisons revealed a substantial transition in gene expression during early VBNC formation followed by fewer changes as time passed. Resuscitation resulted in a significant burst in the gene expression profile distinct from pre-VBNC populations indicating that resuscitated populations take on a unique physiology. Genes highly expressed in resuscitated cells included a number of putative virulence factors suggesting that these cells could be in a hyper-virulent state. Currently, we are investigating specific differentially expressed genes to pinpoint mechanisms underlying the dormancy process. This temporal study will allow for a more resolved investigation into the complex dynamics involved in VBNC cell formation and resuscitation.

The effect of hydration and connectivity on the soil food chain

Adam Stovicek, Osnat Gillor
Ben Gurion University of the Negev, Israel

Soil microbial communities use dormancy to avoid stressful conditions such as extreme temperatures, desiccation or nutrient depletion by limiting their metabolic activity. Conditions could be favorable in a brief event, such as rain in the desert. However this is sufficient to activate the soil microbial community. When the resuscitation period is both abrupt and short lived, the microbial communities are taking full advantage of the brief abundance in resources. This abrupt transfer from dormancy to full activity is experienced by the entire microbial community. However, it was previously described mainly for bacteria, ignoring archaea, fungi, or protists.

Moreover, no study has yet taken a holistic approach when examining changes in soil microbial community in response to rainfall. We predict that rain in the desert increases the soil connectivity, enabling interactions such as predator-prey or trophic competition. To test this hypothesis an RNA shotgun sequencing approach was developed and employed to analyze the entire soil microbial community during a rain event after a long drought. Soil samples were collected in the Negev Desert (Israel) during an annual rain event.

The results suggest that the microbial community significantly changed during the rain event. This change was driven on the one hand by new substrate availability and relief of desiccation stress, and on the other hand by connectivity enabling predation and resource competition. This is evidenced by an increase of predatory amoeba (mainly Acanthamoeba) during the rain event. These antagonistic interactions further alter the microbial community, decreasing the abundance of some phyla (mainly Actinobacteria and Basidiomycota), while increasing the abundance of others (mainly Firmicutes and Ascomycota).

Desiccation and loss of soil connectivity fragment communities and impede interactions and access to substrate thus suppressing the microbial community growth and activity. Consequently, microbial activity in soil ais mostly confined hydration events and demand rapid response from the generally dormant community.

Importance of the pyruvate sensing network BtsSR/YpdAB for the resuscitation of Escherichia coli from the viable but nonculturable state

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Viable but nonculturable (VBNC) cells endure a variety of environmental stresses (e.g., nutrient limitation, pH variations, oxidative stress, fluctuating temperatures) during induction and resuscitation from this state. These external changes need to be sensed and the information transmitted intracellularly, allowing bacteria to respond accordingly. *Escherichia coli* contains 30 two-component systems (TCSs), each composed of a histidine kinase (HK) and a response regulator (RR). The BtsS/BtsR and YpdA/YpdB TCSs have been characterized in more detail in our laboratory (1–3). In *E. coli*, BtsS/BtsR activation leads to the induction of *yjiY*, whereas YpdA/YpdB activates *yhjX* expression. *yjiY* and *yhjX* code for putative transporters. We demonstrated that the HK BtsS binds extracellular pyruvate with high affinity (4). Additionally, we found that the YpdA/YpdB system responds to extracellular pyruvate with low affinity (1).

We tested the effect of these two systems on the resuscitation of E. coli from the VBNC state. We induced the state by exposure of cells to oxidative stress or low temperature. Resuscitation of cells occurred only in the concomitant presence of pyruvate and the BtsSR/YpdAB systems. Furthermore, we performed transport assays and discovered that pyruvate is taken up by the VBNC cells. Wild-type and deletion mutants indicated differences in the time dependent protein and DNA biosynthesis during resuscitation, corroborating the importance of the BtsSR/YpdAB systems. Finally we performed time-lapse microscopy and monitored pyruvate- and BtsSR/YpdAB- dependent resuscitation of E. coli from the VBNC state at single-cell level. Our results demonstrate the importance of sensing and transporting pyruvate during resuscitation of E. coli from the VBNC state.

References:

1-Fried, et al (2013), J Bacteriol 195:807–15; **2**-Kraxenberger, et al (2012), J Bacteriol 194:4272–84; **3**-Behr, et al (2014), J Bacteriol 196:2023–9; **4**-Behr, et al (2017), Sci Rep.

SESSION 3: ECOLOGY AND SURVIVAL

Invited talk

Sleep, stress, and the Black Queen: Microbial hidden activities and cooperation

Holger Daims

Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, University of Vienna, Austria

Cultivation-independent tools to track metabolic activities at the single cell level are highly suitable for monitoring the physiological status of apparently inactive microorganisms. Here the use of single-cell Raman microspectroscopy to illuminate the ecophysiology of 'resting' and starving microbes is demonstrated. First, application of this technique led to the surprising discovery that elementary bodies – the 'resting' stages of environmental and pathogenic *Chlamydiae* – are metabolically active even outside their eukaryotic hosts. This finding has major implications for our understanding of the infection mechanisms and the virulence of these medically important organisms. Second, single-cell Raman analysis provided first insights into the physiological reorganization of non-growing complete nitrifiers ('comammox' organisms) during starvation. In the absence of their primary substrate ammonia, these bacteria exhibit strong shifts in their cellular composition that could be consistent with a versatile, highly economical and oligotrophic lifestyle. Finally, combined genomic and physiological analyses provided new insights into the ecology and biological interactions of ammonia-oxidizing archaea (AOA). Lacking a key stress response and detoxification mechanism after gene truncation and loss, these nitrifiers rely on microbial partners in their neighborhood to survive. This example of the 'Black Queen Hypothesis' is striking as it affects key players in a major biogeochemical cycle, and it explains many of the difficulties to isolate and cultivate AOA and other nitrifying microorganisms.

RpoS-independent evolution reveals the importance of attenuated cAMP/CRP regulation in high hydrostatic pressure resistance acquisition in *E. coli*

Elisa Gayán, Chris W. Michiels, Abram Aertsen

KU Leuven (Department of Microbial and Molecular Systems, Laboratory of Food Microbiology)

High hydrostatic pressure (HHP) processing is an attractive non-thermal alternative to food pasteurization as it inactivates microorganisms without compromising food quality. Nevertheless, the large inter- and intra-species variations in HHP resistance among foodborne pathogens and the ease by which they can acquire extreme resistance are an issue of increasing concern. Since RpoS activity has been considered as a central determinant in the HHP resistance of *E. coli* and its pathovars, this study probed for the potential of an *E. coli* MG1655 *ΔrpoS* mutant to acquire HHP resistance by directed evolution. Despite the much higher initial HHP sensitivity of the *ΔrpoS* mutant compared to the wild-type strain, evolved lineages of the former readily managed to restore or even succeed wild-type levels of resistance. A number of these *ΔrpoS* derivatives were affected in cAMP/CRP regulation, and this could be causally related to their HHP resistance. Subsequent inspection revealed that some of the previously isolated HHP-resistant mutants derived from the wild-type strain also incurred a causal decrease in cAMP/CRP regulation. cAMP/CRP attenuated HHP-resistant mutants also exhibited higher resistance to fosfomycin, a preferred treatment for Shiga toxin-producing *E. coli* infections. As such, this study reveals attenuation of cAMP/CRP regulation as a relevant and RpoS-independent evolutionary route towards HHP resistance in *E. coli* that coincides with fosfomycin resistance.

Low nucleic acid-content bacteria: viable, unique microorganisms

Caitlin Proctor, Frederik Hammes

Eawag – Swiss Federal Institute for Aquatic Science and Technology, Duebendorf, Switzerland

Low nucleic acid (LNA)-content bacteria comprise a dominant cluster of bacteria observed with flow cytometry (FCM). These LNA-content bacteria are small and low in fluorescence after staining of nucleic acids. They have long been contended as dead, inactive, or starved forms of bacteria. While LNA have been shown to use substrate and grow, it is still not clear what this cluster is or why they exhibit such stark separation from other bacteria in FCM signals. We hypothesized that these bacteria were phylogenetically unique bacteria, rather than dead bacteria or a varied physiological state of the same overall bacterial community.

In this study, we used FCM to demonstrate the prevalence of this distinct cluster across five ecosystems (lake water, river water, tap water, wastewater, and groundwater). We linked low fluorescence of LNA-content bacteria measured with FCM to small cell size (e.g. 0.016 µm3), filterability (0.4 µm), and low DNA content. Using filtration we separated LNA-content bacteria from high nucleic acid (HNA)-content bacteria, and identified them with 16S amplicon sequencing. Many individual OTUs could be classified as exclusively LNA (498 OTUs) or HNA (459 OTUs), even across five diverse ecosystems. These OTUs clustered strongly on the phylum level, suggesting that classification as LNA- or HNA-content bacteria is part of a bacterium's fundamental identity and evolutionary history. Many LNA OTUs were identified as candidate phyla that lack many cultured representatives (e.g., OD1, GN02, TM7, TM6, and OP3) and parasitic organisms (e.g. *Bdellovibrio*), suggesting that they have a limited capacity for independent life.

Overall, our data supports previous suggestions that LNA-content bacteria are viable, unique microorganisms relevant to our understanding of microbial communities in aquatic ecosystems. It also suggests that 0.4 µm filtration could be a rough barrier distinguishing free-living independent life.

The viable but non-culturable state in food processing environments

Christian Robben, Peter Rossmanith, Susanne Fister, Dagmar Schoder, Tobias Gundolf, Anna Witte, Patrick Mester

Christian Doppler Laboratory for Monitoring of Microbial Contaminants, Institute for Milk Hygiene, Veterinary University of Vienna, Austria

The accumulation of unfavourable environmental conditions in food processing environments can lead to the induction of viable but non-culturable (VBNC) state. The present study investigates the induction of the VBNC state in *L. monocytogenes*, *E. coli* and *S.* Typhimurium by commonly used detergents and household cleaners in combination with salts.

For induction of the VBNC state we combined several different non-toxic detergents and cleaners with authorised salts, commonly used in food-processing environments. A possible induction of the VBNC state was investigated by measuring metabolic activity and examining the membrane integrity using the LIVE/DEAD *Bac*Light viability assay. For further research prospects, we also analysed the storage ability of these VBNC cells.

We confirmed the VBNC state for all tested bacteria after exposure to different combinations of detergents and commercially available household cleaners with salts. The VBNC state could be confirmed already 20 minutes after induction. The time bacterial cells are exposed to this combination had only little effect on the cells in VBNC state and we were able to store them for at least one week in Ringer solution.

The induction of the VBNC state in foodborne pathogens by a combination of detergents and salts, commonly found in food processing environments is a new, fast and stable VBNC induction method and therefore a great public health concern. However, a fast and stable procedure for induction and storage of VBNC cells offers a plenty of research prospects on the other hand.

VBNC state and 'regrowth' during waste treatment: factors playing a role in loss of culturability of pathogenic bacteria seeded in digestates from agricultural biogas plants

Géraldine Maynaud, Anne-Marie Pourcher, Christine Ziebal, Nathalie Wéry

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During treatment of organic waste by anaerobic digestion, composting or in wastewater treatment plants, pathogenic bacteria are submitted to various biotic and abiotic stress. In treated wastes (digestates, composts…) increase of bacterial counts obtained by culture for indicators (*E. coli*) and pathogenic bacteria (*Salmonella sp., Shigella* sp., *Listeria monocytogenes*) are sometimes recorded, but the underlying mechanisms remain unknown. Some recent studies indicate that this may be due to the induction of Viable but Non-Culturable (VBNC) state during treatment, followed by 'resuscitation' during storage of treated products. Here, a microcosm approach was used to evaluate the persistence of three pathogenic bacteria (*Salmonella* Derby, *Campylobacter* coli and *Listeria monocytogenes*) in digestates from agricultural wastes, stored for later land spreading. Nine samples, including raw digestates, liquid fractions of digestate and composted digestates, were inoculated with each pathogen and maintained for 40 days at 24°C. Concentrations of pathogens were monitored using culture and qPCR methods. In some digestates, the concentration of the pathogens by qPCR assay was several orders of magnitude higher than the concentration of culturable cells, suggesting a potential loss of culturability and induction of VBNC state. The potential VBNC state which was generally not observed in the same digestate for the three pathogens, occurred more frequently for *C. coli* and *L. monocytogenes* than for *Salmonella* Derby. The effect of NH4+/NH3 on the culturability of *C. coli* and *Salmonella* Derby was also shown. Our results underline the importance of considering VBNC cells when evaluating the sanitary effect of an anaerobic digestion process and the persistence of pathogens during the storage of digestates and subsequent land spreading.

SESSION 4: IMPROVEMENTS IN CULTURE-INDEPENDENT DETECTION

Invited talk

Why are cultivation-independent viability methods advancing so slowly?

Frederik Hammes

Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

The last two decades are filled with exciting advances in our ability to detect and characterise bacteria. Powerful cytometric and molecular methods, as well as large strides in the field of single-cell research, have all contributed to a considerable better understanding of bacteria and bacterial processes. Many of these methods are now being automated, allowing high frequency tracking of bacterial viability dynamics in both experimental set-ups and field applications. Despite these positives, there remain numerous challenges. Viability assessment methods for indigenous bacterial communities remain limited, with most of the focus on the detection of severe membrane damage with for example propidium iodide (cytometry) and propidium mono-azide (molecular). While useful, this in turn limits our understanding of the complexities of bacterial damage and death, and severely limits the practical applications for which these methods are useful. This presentation will focus on recent advances in viability methods, highlighting emerging challenges with specific examples, and exploring opportunities for future research and development in this field.

Invited talk

Viability PCR and assessment of UV disinfection efficiency: Amplicon size matters

Andreas Nocker, Laura Seidel, Mili Shah, Julios Kontchou, Martin Strathmann, Gabriela Schaule
IWW Water Center, Applied Microbiology, Moritzstrasse 26, 45476 Mülheim an der Ruhr, Germany

Viability PCR has in recent years received much attention in various fields of microbiological research. It aims at preferential detection of microorganisms that possess an intact cell membrane and are thus potentially viable. Signals from microorganisms with compromised cell envelopes on the other hand are suppressed by treating samples with a viability dye that selectively enters damaged cells and binds to their nucleic acids. Subsequent light exposure leads to activation of the viability dye, which covalently links to their nucleic acids. The resulting irreversible alteration in turn reduces their amplifiability. As a consequence PCR signals mostly originate from intact microbes whose nucleic acids had not been exposed to active dye. We show in this presentation the effect of amplicon length on the estimated ratios of intact cells using Microbial Source Tracking (MST) and specific assays for *Legionella pneumophila and Pseudomonas aeruginosa* as application examples. In MST, use of longer amplicons can greatly reduce the probability to detect DNA from old fecal material and thus contribute to improved risk assessment. Another application where amplicon size is of critical importance is the assessment of the efficiency of UV disinfection. Assuming random distribution of damages, the amplification of longer sequences increases the probability of polymerase stalling in PCR. Targeting segments of different lengths can be used to estimate the UV dose that was actually received by microorganisms upon passage through a UV reactor. Such molecular measurements can thus compliment culture-based biodosimetry.

SESSION 4: IMPROVEMENTS IN CULTURE-INDEPENDENT DETECTION

From Cradle to Grave: On-line and Real-time Tracking of Short-term Microbial Dynamics

Michael D. Besmer[1,2], Frederik Hammes[1,2]

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With bacteria being an integral part of natural and engineered aquatic ecosystems, it is crucial to closely monitor their number and composition. This allows for better understanding and management of bacteria and their impacts. One critical aspect in this is the tracking of microbial dynamics at short timescales (seconds to days) given that those ecosystems are usually highly variable rather than stable over time. Such microbial dynamics span the entire lifecycle of bacteria, including multiplication, exchange between biofilms and planktonic phase, (environmental) stress, and death/inactivation.

Tracking of these dynamics requires sampling and analysis at very short intervals and ideally in real-time allowing for immediate interpretation/reaction. This can only be achieved through full automation of in-situ sampling, sample processing, and detection. For decades this was impossible with conventional cultivation-based methods but also with advanced molecular methods, which are still too labor intensive, time consuming, and costly for such applications. However, in recent years much progress was made on automated monitoring. One promising approach was the automation of flow cytometry. The resultant richness in data of highly resolved time-series allows exploiting the proven advantages of flow cytometry (e.g., rapid, sensitive, reproducible, quantitative, enumeration of total and intact cells, fingerprinting) to a much greater extent.

Today, this technology is at the edge of commercialization and can be expected to rapidly become a standard tool in fundamental and applied research but also various industries. Hence, here we present the scientific bases of the method that were established in the last five years and discuss its potential and limitations. Specifically, we show experimental data on (1) growth behavior of natural bacteria in cell numbers and community fingerprints, (2) short-term detachment dynamics in full-scale water treatment and distribution linked to operational practices, (3) effects of water pollution and disinfection through oxidation on cell numbers and viability.

Rapid detection of Legionella by IMS and flow cytometry

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Legionella is an opportunistic pathogen of high public health concern. It is the causative agent of atypical pneumonia known as Legionnaires' disease as well as the acute febrile illness known as Pontiac fever. *Legionella* is responsible for both nosocomial and community-acquired infections. Potable water systems are the primary source of *Legionella* infections, causing over 50% of all drinking water-related outbreaks, increasingly adding to annual health costs. Currently, the ISO 11731 culture-dependent method is the standard for the isolation and enumeration of *Legionella* from water samples. This method is inaccurate, time-consuming and cannot detect viable but non-culturable (VBNC) bacteria, thus largely underestimating the population numbers of *Legionella* present in water. To solve this problem, we have developed an automated, culture-independent immunomagnetic separation (IMS) method that shortens the time from sample-to-result to 1-2 hours, instead of several days. Using highly specific monoclonal antibodies, we increased the sensitivity from five to ten times over the traditional, culture-dependent method. Combining the automated IMS with flow cytometry, we can accurately enumerate total or viable *Legionella* cells present in water samples, including VBNC. Using this method, we successfully detected *Legionella* in different matrices like tap or bottled water and in complex matrices like cooling tower or surface water.

Determination of *E. coli* growth/survival adhered to clay mineral particles

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Clay minerals, as natural nanomaterials are interesting mainly for their high absorption capacity. Additionally, some antimicrobial properties, have been described, although, the exact mechanism of action is not fully known. This work studied fitness of *E. coli* cells after adhesion to clay mineral saponite in respect to better understanding the mechanisms of interaction between bacteria and saponite. Preliminary results showed an inhibition effect of saponite to *E. coli* isolated from uro-infection and environment using the concentration range from 0.125 to 5 mg/ml. Observed differences among uro-isolates and enviro-isolates of *E. coli* corresponded to the ability of isolates to form biofilm. Additionally, the presence of genes *fimA* and pap encoding surface structure, such as adhesines and fimbriae seemed to be important. For determination of growth/survival of *E. coli* strains three methods were selected: MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) reduction assay, determination of CFU/ml (colony forming units) and microscopic techniques, such as CLSM (confocal laser scanning microscopy) with Resazurine based on staining viable cells (Almar Blue, 0.02 % w/v) and FISH (fluorescence *in situ* hybridization) with fluorescently labeled rRNA-targeted oligonucleotide probes. Each method provided some information about physiological status of *E. coli* strains. The tendency of higher values achieved by MTT assay in comparison with the CFU assay indicated that cell were bound, but still expressed metabolic activity. CLSM using Resazurin did not significantly proved proportion between live/dead cells adhered to saponite because of itself binding to saponite particles. Finally, FISH method confirmed viability of attached cells on the saponite surface. Moreover, huge deposits of death cells in the deeper layers of clay were observed after 22 h. We suppose that surface structure of bacteria significantly contribute to adhesion to saponite, but physical capturing mechanism involving electrochemical interactions of bacterial cell and clay surface seems to be important in definitive damage of bacteria.-

Microbial community fingerprinting of water resources by flow cytometry

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Flow cytometry is a relatively novel tool in the field of water quality assessment, despite being in use by medical research and clinical practice for several decades. In 2012, the Swiss Federal Office of Public Health acknowledged the usefulness of the method and recommended it to be applied for total bacterial cell counts and ratios of high and low nucleic acid content cells in a comparative manner. The application of flow cytometry for water resources introduces specific challenges, such as the unknown composition of the bacterial community in different types of groundwater or the dynamics of these communities to environmental changes. Further, these environmental samples pose challenges due to their biogeochemical characteristics.

The here presented work aims to adapt the analysis of flow cytometric data in order to fulfill the needs of research as well as those of the practitioners at the waterworks. A special regard is given to time series of flow cytometric data as part of an extended measuring program. On this basis, an enhanced insight into the stable or dynamic nature of a selection of Austrian groundwater bodies at the point of extraction as related to flow cytometry is possible. This insight is fundamental for recommending on the practical usage of flow cytometry under specific hydrogeological conditions and for integrating flow cytometry into the established scheme of measurements. On this behalf not only flow cytometric measurements are used but it is crucial to widen the focus to already available data and established methodologies.

SESSION 5: REGULATION OF SLEEP AND DEATH

Invited talk

Fitness and Survival in Mycobacterium tuberculosis dormancy

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Mycobacterium tuberculosis is to date the most prevalent infectious disease. Worldwide a third of the population is estimated to be infected, though in most cases *M. tuberculosis* persists in a state of reduced metabolic activity where it does not replicate and remains symptomless. During that time, it is tolerant to antibiotics and evades host immune defenses but it also retains the ability to proliferate, which allows it to cause active disease at any given time. Therefore, understanding the mechanism of persistence in *M. tuberculosis* under the stresses that are imposed by the immune system or antibiotics is essential to treat tuberculosis. We attempt to find and characterize those factors using the in vitro carbon-starvation model that renders *M. tuberculosis* antibiotic tolerant and is considered a proxy to the non-replicating or latent state in vivo. To that end, we performed high throughput genomic screens and Transposon-sequencing using a transposon mutant library that covers the entirety of the non-essential genome. Among the most interesting hits was an mce associated protein that is conserved among many Mycobacterial species. A deletion of the gene decreases fitness, its over-expression enhances survival dramatically during carbon-starvation. In combination with transcriptome profiling (RNA-seq) we try to decipher its mechanism of action. In the long term, we hope these findings can contribute to a better understanding of the non-replicating state in *M. tuberculosis* and have the potential to improve or shorten the very long tuberculosis therapy in the future.

Deterministic heterogeneity drives differential heat inactivation within clonal bacterial populations

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Even under homogeneous environmental conditions isogenic cells within a clonal bacterial population tend to display intercellular differences that can result in differential behavior [1]. An important example of such phenotypic heterogeneity is the typical observation that not all cells within a clonal population are equally sensitive to a stressful encounter, with the underlying reason or mechanism often being elusive. However, when using time-lapse fluorescence microscopy to scrutinize individual cellular survival odds in an isogenic population of the low infectious dose foodborne pathogen *Salmonella* Typhimurium before and after heat treatment, we surprisingly found the size of unstressed *S.* Typhimurium cells to be quite variable and to closely correlate with cellular fate after heat shock. In fact, smaller viable cells pre-existing within the clonal population clearly displayed a lower probability of surviving a heat shock, and we were able to link both phenotypes to the inherently bistable expression of S. typhimurium virulence factors. As such, while differential inactivation within clonal bacterial populations is often thought to stem from the unpredictable stochastic variation of cellular attributes, our results underscore the importance of more deterministic biological differentiation events as well.

[1] Ackermann M. (2015). A functional perspective on phenotypic heterogeneity in microorganisms. Nat. Rev. Microbiol. 13:497-508.

Proteolysis of virulence regulator ToxR mediates entry of Vibrio cholerae into a viable but nonculturable state

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Vibrio cholerae PG1 is a natural inhabitant of aquatic environments and causes the diarrheal disease cholera. Two of its primary virulence regulators, TcpP and ToxR, are localized in the inner membrane. TcpP is encoded on the *Vibrio* Pathogenicity Island (VPI), a horizontally acquired mobile genetic element, and functions primarily in virulence gene regulation. TcpP has been shown to undergo regulated intramembrane proteolysis (RIP) in response to environmental conditions that are unfavorable for virulence gene expression. ToxR is encoded in the ancestral genome and is present in non-pathogenic strains of *V. cholerae*, indicating it has roles outside of the human host. In this study, we show that ToxR undergoes RIP in *V. cholerae* in response to nutrient limitation at alkaline pH, a condition that occurs during the stationary phase of growth. This process involves the site-2 protease RseP (YaeL), and is dependent upon the RpoE-mediated periplasmic stress response, as deletion mutants for the genes encoding these two proteins cannot proteolyze ToxR under nutrient limitation at alkaline pH. We determined that the loss of ToxR, genetically or by proteolysis, is associated with entry of *V. cholerae* into a dormant state in which the bacterium is normally found in the aquatic environment called viable but nonculturable (VBNC). Strains that can proteolyze ToxR, or do not encode it, lose culturability yet remain viable under nutrient limitation at alkaline pH and also experience a change in morphology associated with cells in VBNC. On the other hand, strains that cannot proteolyze ToxR remain culturable and maintain the morphology of cells in an active state of growth. Overall, our findings provide a link between the proteolysis of a virulence regulator and the entry of a pathogen into an environmentally persistent state.

Characterization of a chromosomal toxin-antitoxin module conserved in *Nitrosomonas europaea*

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Background

Toxin-antitoxin (TA) systems are small genetic modules that regulate microbial cell growth under stressful conditions. Recently, several reports showed that these TA pairs are likely to be abundant in some chemolithotrophic prokaryotes. *Nitrosomonas europaea*, a well-known ammonia-oxidizing bacterium, is one of such representative examples. Of note, N. europaea is predicted to have more than 50 TA pairs in its chromosome; thus, *N. europaea* may use these TA pairs to overcome versatile environmental stressors.

Objectives

We sought to characterise one of the representative TA systems, MazEF, which is composed of a MazF toxin along with a MazE antitoxin. MazF toxins are, in most cases, sequence-specific endoribonucleases; therefore, we purified a putative *N. europaea* MazF endoribonuclease and tried to determine its target sequence.

Methods

RNA substrates were fragmented by the MazF endoribonuclease, and the cleaved RNA sites were analysed by a specialised RNA-Seq method. Next, short oligonucleotides containing specific sequences inferred to be prerequisite for MazF cleavage were prepared and incubated with MazF. The cleavage activity as a function of time was monitored by a fluorometric assay.

Conclusions

We found that *N. europaea* MazF is functional and forms a genuine TA pair with its cognate antitoxin MazE. In addition, we revealed that the MazF toxin is a three-base cutter. Our results suggest that *N. europaea* alters its translation profile and modulates its growth using MazF in certain stressful environments.

The role of stress-related saoABC operon in intracellular survival and persister formation in Staphylococcus aureus

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Stress response systems provide a bacterial cell with the necessary plasticity required for living in dynamically changing environmental conditions. Within these, such conditions include biologically relevant interactions between bacteria and a human host. Next to a few well-characterised regulatory systems in staphylococci, there are those which remain to be explored. Nonetheless there is an even greater need to better understand the link between such systems and the phenomenon of persister formation.

This study aimed at a comprehensive analysis of a hypothetical *saoABC* operon, which likely constitutes a gene expression regulatory system, and its role in persister formation as well as in survival of bacteria internalised into the host cells.

The research provided an insight in *saoABC* operon structure and conservation among species of *Staphylococcus* genus. Next to a clear demonstration of DNA-binding properties of *SaoC* protein, the results show the changes in expression of *saoABC* genes triggered by a particular set of stress stimuli related to bacteria-to-host internalisation, which are starvation and acidification. A strong link between *saoABC* operon and known gene transcription regulatory systems is demonstrated. Growth rate decrease in nutrient-limiting conditions as well as a significant change in persister formation was observed for *ΔsaoB* mutant next to decreased intracellular survival of *ΔsaoC* one.

The results of the study convincingly suggest the existence of a strong link between the functions of *saoABC* operon and the stress response. The changes in expression of *saoABC* genes as well as the phenotypes of *ΔsaoC* and *ΔsaoB* mutants point out the possible importance of *saoABC* operon in phenomena of intracellular survival of the pathogen and persister formation.

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SESSION 6: CLINICAL CONSEQUENCES OF SLEEPING PATHOGENS

Invited talk

HDID in Biofilm associated infections? Diagnostics, complications and consequences

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Biofilm-associated infections pose a significant risk for patients, since they tolerate higher concentrations of antibiotics than measured as minimal inhibitory concentrations (MIC). Standard clinical diagnostics comprise cultivation and testing for antibiotic susceptibility. However, these tests imply disintegration of the biofilm and growth of the bacteria in vitro. Therefore, the routine procedures might miss bacteria in stationary phase, presumable persister cells and give no information about spatial distribution of the viable cells or active biofilm layers.
We use Fluorescence in situ hybridization (FISH) to diagnose biofilm-associated infections. To detect the activity of single bacterial cells more precisely, we developed FISH probes for the 16S-23S internal transcribed spacer region that is only present in actively transcribing cells. Using this spacer-FISH technique we detected positive cells in heart valves of patients who had been given adequate therapy. We furthermore used FISH for in vitro susceptibility measurement of biofilms.
These findings confirm the recalcitrance of bacteria towards antibiotic treatment in biofilms in the clinical setting. They stress the point that our current diagnostic techniques for cultivation and antibiotic resistance testing in vitro are not satisfactory. In situ techniques are able to translate findings from the benchside to in vivo grown biofilms in clinical samples.

Invited talk

How Dead Is Dead in Biomaterial-associated Infection?

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Inserted or implanted medical devices, collectively referred to as biomaterials, save many lives and provide quality of life, but they carry a risk of infection. Staphylococci are the major causative agents of these biomaterial-associated infections (BAI). They can form biofilms on the biomaterials and can also persist within host phagocytes in the tissue surrounding the biomaterials, since immunity locally is compromized. In the EU project BALI, Biofilm Alliance, we have developed novel Synthetic Antimicrobial and Antibiofilm Peptides to prevent and treat BAI. The structures of these SAAPs were inspired by the native human antimicrobial proteins Thrombocidin-1 and LL-37. The development, in vitro activity, in vivo efficacy, and mode of action of these novel highly promising antimicrobials will be presented. This study was supported by FP-7-HEALTH-2011 grant 278890, BALI.

Determination of reversion frequency of clinical Thymidine dependent Small-Colony Variants of *Staphylococcus aureus*

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Introduction:
Staphylococcus aureus Small Colony Variants (SCVs) are known for being a strategy of adaptation to hostile environments. *S. aureus* SCVs are able to persist in eukaryotic cells throughout the course of antibiotic therapy and subsequently colonize the host extracellularly by reverting back to the wild type phenotype. This phenotype switching is based on the basal mutation rate of the strain and the type of mutation that led to the SCV phenotype. In this study, we characterized the reversion frequency of clinical Thymidine Dependent Small Colony Variants (TD-SCVs) of *S. aureus* from the SCV phenotype to the wild type phenotype.
Methods:
Sequence analysis of twelve TD-SCVs isolated from Cystic Fibrosis patients was performed with reference to a previously published *S. aureus thyA* nucleotide sequence to determine the mutational cause of their thymidine dependency. Selected TD-SCVs were then analyzed for their frequency of reversion to the wildtype phenotype in a new experimental setting.
Results:
All TD-SCVs tested for their reversion frequency showed a quite diverse ability to revert back to the wild type phenotype, ranging from up to 90% of revertants to 0%. The frequency of reversion was not solely dependent on the primary mutations that led to the SCV phenotype, but also on the genetic background of the strain.
Sequence analysis of *thyA* showed that the SCV mutations occurred at various sites throughout the entire gene. Secondary mutations resulted in revertants that presented the wild type sequence but also presented mutational scars within the sequence.
Discussion:
Concluding, the ability of TD-SCVs to revert did not only depend on their primary mutations in *thyA*, which led to their thymidine dependency, but also on other factors. We suggest that this is due to the different genetic backgrounds of the strains, resulting in a different reversion frequency.

Viability of *Legionella* during short and long time starvation

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Under adverse conditions legionellae switch to a dormant form and get non-culturable on standard media. They may be resuscitated by intracellular growth in host-organisms. Whether this viable-but-non-culturable (VBNC) form of legionellae is of health relevance is not known. To better understand VBNC legionellae, we established model systems to induce and characterize the transition from the culturable to the VBNC state of five *L. pneumophila* and one *L. micdadei* strains. Cells were starved in ultrapure water at 45°C. They were monitored with respect to culturability, viability and virulence potential (binding affinity of antibodies against surface virulence-markers) until 2-3 weeks after complete loss of culturability in more detail and for one year at sporadic sampling-events. *L. pneumophila* serogroup 1 strains became completely non-culturable latest after two weeks, the other strains showed culturability for longer periods. In parallel, viable cell numbers of the *L. pneumophila* strains were reduced initially by 1 log unit. After complete loss of culturability the remaining viable part of the population showed stable signs of viability in terms of esterase activity and membrane integrity throughout the short-time phase. Among the starving *Legionella* populations different sub-populations dynamically developed as observed by flow cytometry; i.e cells with high and low esterase activity. The incorporation rate of leucine was reduced below the quantification level until all cells had lost culturability. Most of the membrane bound LPS-and protein structures tested were expressed at a high level until 200 days of starvation. After 200 days, there was a second drop in viable cell numbers, the VBNC cells started to die. Nevertheless, a few even highly esterase active cells remained. All these data indicate that a certain part of starved *Legionella* populations enters the VBNC state constantly expressing important virulence markers for more than half a year. It thus cannot be excluded that starved legionellae are of relevance to human health.
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Infectivity potential of VBNC Legionella strains on amoebae and human macrophages produced under starvation conditions

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The proliferation of *Legionellae* in engineered water systems is related to the interaction with amoebae, which provide nutrients for the intracellular bacteria and a shelter against harsh environmental conditions. Under unfavourable conditions *Legionellae* enter into a viable but not culturable (VBNC) state in which the bacterial cells are unable to form colonies on standard medium but are still alive.The aim of the current study was to assess the infectivity potential of artificially produced VBNC *Legionella* spp. cells to amoebae and human macrophages. We induced the non culturable state of six *Legionella* strains by starvation at 45°C in ultrapure water. The transition to such state was monitored by using different viability markers. In this work we focussed on monitoring the replication of these *Legionella* spp. strains within an Acanthamoeba strain a human macrophage-like cell line (THP1) and human macrophages.Samples were taken weekly for the first five weeks and at three different later time points (>100 days) and co-cultured with the three hosts using different bacteria:host ratios. Results showed a high variability between the strains tested regarding their replication within the amoebal hosts. Most of the strains replicated within amoebae the first week after the loss of culturability. After longer times under starvation conditions, replicating bacterial cells were observed for some strains only at the highest bacterial concentration used. Remarkably, most of the VBNC *Legionella* spp. strains investigated did not replicate within THP1 cells throughout the five weeks of starvation and only few in later time points. However, some VBNC *Legionella* spp. strains infected human macrophages after 200 days under the same conditions. Thus, starved VBNC *Legionella* cells may represent a risk for human health since they were able to replicate within host cells although they could not be detected by cultivation on standard medium.

POSTER PRESENTATIONS

POSTER 1

Single-cell level activity measurements in terrestrial ecosystems using heavy water combined with Raman microspectroscopy and high-resolution secondary ion mass spectrometry (NanoSIMS)

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Soils encompass a large area on Earth, thereby significantly contributing to global biogeochemical cycles. These cycles are driven by soil microorganisms, however, many of them are in a dormant state at a given time. Interestingly the activity patterns of the soil microbial communities can differ dramatically in different systems. For example, it is estimated that only ca. 20% of the microbial community is active in temperate soil. In contrast, drylands exhibit short phases of high microbial activity, as the microbial community is forced to enter a dormant state to survive periods of low water availability. As such investigating the active microbial communities is challenging not only due to activity patterns, but also due to dispersed cells within a high soil particle background. We have developed and optimized a pipeline to investigate active microbial members in these differential active soils using stable isotopes in combination with Raman microscpectroscopy and high-resolution secondary ion mass spectrometry (NanoSIMS). In order to not change the substrate pool, as is often the case when using 13C or 15N-labeled substrates, we have used 18O- or deuterium-labeled water (D2O) as general activity markers. Anabolic active cells were identified in temperate soils using D2O combined with Raman microspectroscopy. Dryland soils are covered to a large extend by photosynthetically active surface communities, biological soil crusts (BSCs). Our single-cell pipeline is used to reveal short-term resuscitation dynamics on the single-cell level over time in these BSCs along with factors influencing resuscitation. By combining these methods with other molecular tools such as fluorescence in situ hybridization, we can also identify resuscitation and activity patterns of specific microbial community members.

POSTER 2

High selection or high recovery – enumeration of culturable *Legionella* isolates on solid nutrients and demonstration of non-culturable bacteria

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The commonly used method for *Legionella* spp. detection in environmental water samples is culturing on solid selective media. Bacteria are cultivated by streaking on agar plates and the numbers of living cells grown on nutrient plates are quantified by colony counting. To determine bacterial numbers in any given water sample techniques for concentration are performed as filtration through filters in at least 100 ml volumes followed by cultivation and additional immediate plating of 2 x 0.5 ml water sample. However, the real number of *Legionella* spp. may be decreased when high numbers of concomitant cells are present in the water probe and, additionally, the recovery is low when the nutrient is high selective. In this case low recovery may indicate stress for bacteria followed by limitation of growth but existence of living cells.

In this study we analyzed the influence of sample pretreatments and of the selection by nutrient amendments on bacterial recovery on plates. *Legionella* spp. isolates were cultivated on GVPC and BCYE agar plates according to ISO11731. To reduce concomitant bacteria water samples were pre-treated with heat (50°C for 30 min) or with acid (pH 2.2). Both pre-treatments were compared with an untreated control to analyze influence on the recovery of living cells. In order to estimate the number of real living cells we analyzed the bacteria in water samples with other techniques as quantitative PCR using PMA as indicator for membrane integrity. The number of living cells, estimated via plate counting, decreased by >30% after heating but less with acid treatment (>10%). However, the number of culturable cells increased when high numbers of concomitant bacteria were limited by acid. We found a clear increased number of bacteria on low selective plates compared to the number on high selective nutrients, mainly with *Legionella* non-*pneumophila* isolates.

POSTER 3

Formation of nonculturable *Salmonella enterica typhimurium* isolated from different habitats

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It is well-known, that speed of formation of nonculturable cells is strain dependent. Therefore, our goal was to assess influence of preincubation of *S. Typhimurium* 79 in different substrates on this process. RP4 plasmid was inserted into *S. Typhimurium* cells that had resistance genes for ampicillin, kanamycin. Cells were grown on nutrient broth and then transferred into samples of different substrates: soil (S), tap water (W), minced chicken (P), fresh vegetable salad (V) and nutrient broth - control (C). Preincubation period was 7 days. After that Salmonella was isolated from substrates on nutrient agar with 100μg/ml of ampicillin and 50μg/ml of kanamycin and cells were transferred in artificial seawater (ASW). Flasks with ASW were incubated stationary in the dark at room temperature for 8 months. Samples were taken to assess viability (with Live/Dead®) and culturability. Total cell counts remained relatively constant during the entire incubation time with viability levels close to 100% for all populations. After 3 weeks the lowest culturability was observed for variant P, i.e. culturability lowered by 3 log units. In other populations CFU/ml dropped by 2 orders of magnitude. After 9 weeks in population S, only singular colonies grew. Within first 3 months of observation control population had highest CFU/ml values. After 3 months of incubation in stressful conditions cells of substrain S started exiting the VBNC state – CFU/ml increased but remained the lowest among all populations. By 8 months the highest CFU/ml value was observed for the population W. Obtained data suggest, that preincubation in various substrates prior to entering stress conditions influences speed of shifting into nonculturable state of *S. enterica Typhimurium* 79 and soil has the greatest impact. However in time cells adapted to artificial seawater and numbers of CFU/ml stabilized. Existing in stressful conditions without aeration did not lead to cell death.

Detection of viable and dead bacteria by flow cytometry and luminescence microscopy

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The methods used for calculation of bacteria in populations, have to precisely estimate quantity of viable/dead cells. On the base of these techniques, the number of viable but nonculturable (VBNC) cells are defined. Results of a classical method of viability determination (CFU/ml) are often understated. The aim of experiments was comparison of data on definition of quantity of the viable/dead bacteria received, by means of luminescent microscopy and a flow cytometry. 19 batches of the Russian lyophilized probiotics from different manufacturers were studied. Preparations were coded and the alphabetic reference corresponded to the bacteria name in probiotics (*E.coli*-CB, *Bifidobacterium*-BB, *Lactobacillus*-LB, *Bifidobacterium*+ to *E.coli*-BFC). Flow cytometry was carried out on the Cytomics 500, Coulter Beckman at wavelength of 510-550 nm for viable cells and of 603-623 nm for dead bacteria. Luminescent microscopy was carried out on "Opton" microscope. Preparations were analyzed after staining of cells with Live/Dead® kit. Result expressed in numbers of the viable – green and dead – red bacteria.

Comparison of the results obtained by two methods testifies to high correlation for all data (0,845) irrespective of the manufacturing enterprise and expiration dates. Probably level of preservation of cell viability depended on the production technology. It was revealed that the quantity of viable cells in preparations with the expiration date in 2012 exceeded 80%. One of lactobacilli preparations, stored 20 years, issue in 1993, differed on the number of viable cells from the similar preparations stored of 3 years, for 21%. It is revealed that the quantity of viable bacteria in probiotics decreased by 14 – 21% in 3–6 years of storage, and at longer storage –for 25–30%.

Thus, results of assessment of the viable/dead cells received by two methods revealed high correlation (0,845). Both methods are equivalent for the VBNC detection.

POSTER 5

Spatio-temporal dynamics of dormant cells in Pseudomonas aeruginosa biofilms based on bacterial growth activity

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Biofilm formation in human body causes chronic infections. Among other factors, chronicity originates from dormant cells, which are antibiotic tolerant owing to their non-dividing state and capable of resuscitating after antibiotic removal. Although their detection in complex biofilm environments is challenging, understanding when and where dormant cells arise is crucial to eradicate biofilms effectively.

We previously developed an individual-based multidimensional biofilm model and predicted dormant cell formation against environmental stresses such as nutrient or oxygen limitation. Here, a FRET-based filamenting temperature-sensitive mutant Z (FtsZ) marker capable of visualizing bacterial growth activity was introduced into *Pseudomonas aeruginosa* PAO1. The objective was to elucidate dormant cell dynamics within biofilms and to validate simulation results.

To isolate dormant cells, cyan (CFP) and yellow fluorescent protein (YFP) were fused at the N- and C-terminal domains of the bacterial cytoskeleton FtsZ protein, respectively. Whereas FtsZ monomers are bound to the cell membrane, polymerization occurs in the cytosol during cell division. Using the engineered marker strain, colony biofilm formation was induced and dormant cell localization was characterized in the presence or absence of antibiotics.

When the biofilm thickness was below 30 μm, CFP fluorescence appeared at the top of the biofilm. Intriguingly, when the thickness was over 30 μm, CFP fluorescence shifted from the top to the bottom of the biofilm. As expected, CFP-emitting cells survived despite antibiotic treatment, which indicated that they were in the dormant state. These results suggest that dormant cell localization depends on biofilm thickness with different nutrient and oxygen concentration profiles. We expected that cells at the bottom of thin biofilms consumed nutrients, with cells at the top turning into dormant cells because of nutrient depletion, whereas cells at the bottom of biofilms grown to have certain thickness turned into dormant cells because of oxygen depletion.

POSTER 6

Pseudalert®: A possible plate count independent screening tool in *Pseudomonas* aeruginosa VBNC detection?

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The monitoring of the existence of human bacterial pathogens as *Pseudomonas aeruginosa* and their control are important steps in infection prevention in healthcare. For detection of living and culturable cells the inoculation on agar plates following cultivation and cell growth is used as "gold" standard in routine diagnostics. On the one hand the plate count method is highly sensitive, but depends on the plated bacterial volume, the dilution factor and the medium used. On the other hand this method includes high consumptions in time, resources and manpower. Once bacterial growth is detected, a wide field of xenobiotics are used for disinfections. Although in treatment assays the application of xenobiotics reduces the number of living cells, the agents may induce a living but not culturable status (VBNC) of the bacteria following no or partial growth on plates. An alternative screening system to the plate count method after xenobiotic treatment may be the detection of *Pseudomonas aeruginosa* in a chromogenic liquid assay as the Pseudalert® kit from IDEXX. In this study, we processed the Pseudalert® kit as an assay on microplate level for *Pseudomonas aeruginosa* living cell and VBNC screening process. On microplate level, commonly the measurement of the optical density (OD) or viability assays like WST-8/MTT-test are used additional or instead to plate counting for detecting living cells. These used tools allow rapid detection and are resource efficient but have limitations in verifying active growth and species specificity compared to plate count method. The Pseudalert® kit is commonly used routinely for detection of *Pseudomonas aeruginosa* in drinking water systems displaying a detection limit of 1 cfu in max. 250 ml water according to descriptions of the company. The mode of action based on UV-fluorescent quenched substrate hydrolyses form an enzyme that is produced only in active growing *Pseudomonas aeruginosa* species. In our study we demonstrated species specific active growth detection on microplate level up to >10 cells per well using the Pseudoalert® assay. The kit using on microtiterplate level may be a sensitive, time- and resource-saving alternative technique for *Pseudomonas aeruginosa* detection and VBNC screening.

POSTER 7

Cultivation-independent diagnostics of microbial contamination of explanted knee and hip prostheses from sonication fluids

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A low but increasing percentage of patients with knee and hip prostheses experience systemic inflammation that may be caused by bacterial colonization of the prostheses. After explantation, these prostheses are routinely controlled for bacterial contamination with traditional culture based methods to identify the causative pathogenic organism. For this purpose the explanted prosthesis is submerged into physiological saline solution (sonication fluid) and treated with ultrasound to loosen and remove potential bacteria from the surfaces. The sonication fluid thus eventually contains bacteria, but also cell remnants of the patient (blood cells, tissue cells). In many cases classical microbiological culture based approaches lead to negative results. We therefore aimed to visualize bacteria in the sonication fluid with epifluorescence microscopy and to detect and identify them with 16S-rDNA PCR and sequencing in order to obtain a more comprehensive picture of the bacterial contamination of explanted prostheses. In the presented study we show first results obtained by the two culture-independent methods in comparison to the traditional cultivation based approach and discuss the methodical difficulties encountered during the establishment of epifluorescence microscopy and 16S-rDNA PCR protocols.

POSTER 8

Desert petrichor: volatiles organic compounds emission from desert soil and their relation to microbial communities resuscitated by rain

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To avoid the harsh desert conditions, soil microbial communities are limiting their metabolic activity and are mostly dormant. This dormancy is interspaced by infrequent and unpredictable rain events that alleviate the water stress and resuscitate the dormant microbial community. The revival of the desert soil microbial community results in distinct earthy smell that was shown to be produced by certain bacteria, mainly actinobacteria. Yet, the origin of the petrichor is unclear. We predicted that resuscitation of the biocrust microbial communities is that main source to volatile organic compounds (VOCs) emission while the bulk soil play a secondary role. We further hypothesize that VOCs would reduce along the aridity gradient, as the biocrust community might be less likely to exhaust precious resources for VOCs emission upon hydration. To test these predictions, samples were collected along arid soil horizon (including biocrust, 1-5 cm in depth) and along aridity gradient ranging from 200 to 50 mm of annual rain. The samples were hydrated and VOCs emission monitored using Headspace Solid-Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC/MS). Surprisingly, a limited number of VOCs were detected in the different soil samples, and the majority of profiles were dominated by geosmin and 2-methylisoborneol. The semi-arid and arid biocrust yielded similar profiles while hyper-arid VOCs emission was limited suggesting that spatial emission scale. The results suggest that desert petrichor originate mainly from the hydration and resuscitation of the soil biocrust communities in semi-arid and arid environments. However, the VOCs producer(s) are still unknown. The connection between the resuscitation mechanisms and these VOCs emissions, in addition to the ecological role these metabolites might play in desert soil communities are the subject of this study.

POSTER 9

Fermentation pulse expression leads to formation of *E. coli* persister

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When bacterial population is exposed to antibiotics, only small subpopulation could survive and cause for the recurrence of infections. Persisters are different from antibiotic-resistant mutant, because their tolerances arise from phenotypic variants. Cells which express persister gene stochastically can switch to persister states. Although previous studies identified many interesting genes and phenotypes, molecular mechanisms of bacterial persistence remain unclear because of their redundancy. In order to identify novel persister genes, we developed a marker for *E. coli* persisters and performed transcriptome analysis of isolated persisters.

The objective is to reveal molecular mechanisms of persister formation via lactate dehydrogenase (*ldhA*) which was identified from our transcriptome analysis.

Transcriptome data suggested expression of anaerobic respiration genes despite aerobic cultivation. We screened overexpression and knockdown strains derived from *E. coli* MG1655 at exponential phase. CRISPR interference was used for gene knockdown. To visualize *ldhA* expression, YFP was cloned into pSC101 vector containing *ldhA* promoter. The YFP fluorescence of reporter strain was analyzed by a microfluidic devise.

ldhA overexpression increased ofloxacin persister 100 times, and knockdown decreased the population 10 times in LB medium. Time-lapse microscopy images of *ldhA* reporter strain showed the most of cells did not express *ldhA*, but a few (~1%) cells highly expressed *ldhA*. These cells stopped dividing and tolerated lethal concentration of ampicillin. Interestingly, although *ldhA* was expressed transiently, the cells showed dormant phenotype over 1 hour. These results suggest that stochastic expression of *ldhA* triggers persister formation. We also revealed *ldhA* overexpression broke the balance of NADH/NAD+, and addition of KCN, which inhibits all cytochromes, canceled persister increase via *ldhA* expression. Aerobic respiration may be important for the persistence mechanism.

POSTER 10

Bacterial viability assessment: advantages and challenges of using different methods for monitoring bacterial agony

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Monitoring bacterial agony is challenging and depends on the cause and process of bacterial death, the time point of agonal state, and the target of the viability assessment method. There is currently no 'silver bullet' method, as the different methods address different aspects of viability and death. Here we demonstrate these challenges with a supposedly straightforward heat-disinfection experiment. An E. coli culture was discretely exposed to increasing temperatures from 30 – 65°C. We used three different methods for monitoring agony and death: (1) flow cytometry (FCM) with staining for membrane integrity, (2) cultivation to assess the ability to proliferate, and (3) ATP for measuring metabolic activity. Critically, the influence of small methodological modifications on the experimental outcome and data interpretation was investigated, using EDTA together with FCM and measuring extracellular ATP. We observed clear differences in the results obtained with the different methods: FCM showed intact bacterial membranes until 45°C and complete membrane damage and cell death at 55°C. Importantly, FCM showed no cell damage whatsoever without the addition of EDTA. Cultivation showed a gradual decline in viability until 50°C and complete death from 55°C onwards. In contrast, total ATP initially increased dramatically, followed by a rapid decrease at 55°C, with around 10% remaining as extracellular ATP. This basic experiment showed the importance of understanding both the process of bacterial death and the mechanistic action of viability assessment methods. It highlights the need to use multiple methods in concert to ensure correct interpretations when monitoring bacterial agony and death.

POSTER 11

Estimation of dead and live bacteria ratio using Bacteria Activity monitoring by measuring on-line the alkaline phosphatase concentration

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The BACTcontrol, a microLAN instrument, measures on-line bacterial enzyme activity in food and aquatic samples. Beside ß-D-Glucuronidase, ß-Galactosidase and ß-Glucosidaseto measuring E. coli, Coliform and Enterococci activity respectively also alkaline phosphatase (ALP) activity is measured to determine total activity.
This paper describes the on-line analysis of the total activity based on alkaline phosphatase measurements. A comparison is presented with plate count, flow cytometric and ATP analysis. The results are obtained from the European project Aquavalens, a consortium with SMEs, Industries, Universities and Research Institutes with the mission of protecting the health of European citizens from contaminated drinking water and water used in food processing. Aquavalens will achieve this by developing sustainable technologies to enable water system managers whether in large or small water systems or within food growers or manufacturers to better control the safety of their water supplies.
The benefits of (near) real-time measurements are addressed by providing examples of food and water (drinking-, surface and wastewater) quality monitoring.
In addition a hypothesis is discussed to use the general known principle of alkaline phosphatase activity measurements for the estimation of the dead/live ratio of bacteria by adapting the measurement sequence. In this way it might be possible to estimate a dead/live ratio within 30-60 minutes after sample taking.

POSTER 12

Detection of Campylobacter in Milk by Viability Real-Time PCR

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With 229 213 human cases in 2015 campylobacteriosis continues to be the most frequent bacterial food-borne disease in the European Union. The main causative agent is *C. jejuni*, which is mostly transmitted via contaminated poultry products. However, *Campylobacter* outbreaks may be caused by the consumption of raw cow's milk implying an increasing problem after the European milk quota regime came to an end and farmers intensified the sale of raw milk via milk filling stations. With regard to the detection and quantification of pathogens, milk displays a particularly challenging food matrix due to its high protein and lipid content. Additionally, it has been repeatedly shown that cfu determination underestimates not only cell viability but also the pathogen's potential to cause infections.

We have developed a procedure that allows real-time PCR based detection of viable *Campylobacter* in raw cow's milk. Various DNA-intercalating dyes were evaluated for their ability to reduce the non-viable signal during real-time PCR. Only PMA and PMAxx qualified for diagnostic use. Entry of these dyes into H2O2-killed bacteria depended on cell membrane integrity and passive exclusion was observed with bacteria, transiently inactivated in the presence of the protonophore CCCP but able to regain their colony-forming ability after stress release. The procedure displays a further preferential exclusion of non-viable *Campylobacter* due to different sedimentation properties of live and dead bacteria. Application of the method showed that while various strains of *Campylobacter* significantly lost their colony-forming capacity, the number of viable bacteria detected by real-time PCR remained high, even after 6 days of aerobic incubation at 4 °C. In future studies we will focus on the re-evaluation of *Campylobacter* survival in raw milk with emphasis on the biological relevance of PCR-based viable counts which may (at least in part) be supported by resuscitation experiments.

POSTER 13

Small colony variants: A 'third lifeform' of Bacillus cereus

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Bacillus cereus, a gram-positive, endospore forming bacterium, gains increasing medical relevance as a causative agent of food intoxications and infections, but also of non-gastrointestinal infections. Strains producing the emetic toxin cereulide are of special concerns, as severe intoxications including fatal cases have been increasingly reported in the last years.

Major challenges are hereby the failure of antibiotic therapy, leading to resistant bacteria, and the misdiagnosis of *B. cereus* in clinical settings, resulting in partly severe systemic infections. Within this frame, a panel of emetic *B. cereus* strains was screened for antibiotic resistance patterns. Amongst the tested antibiotics, the aminoglycosides amikacin and gentamicin led to the induction of small colony variants (SCVs), which have been widely described in non-sporulating bacteria, especially Staphylococcus aureus, and proposed as a model for a persistent life style. Importantly, SCV *B. cereus* subpopulations also emerged during amikacin pressure *in vivo*, using a *Galleria mellonella* infection model.

Our work shows for the first time that the spore-forming pathogen *B. cereus* is able to switch to a so far unreported slow-growing lifestyle. The *B. cereus* SCVs substantially differed in their phenotypic, metabolic and virulence traits. In the *G. mellonella* infection model, the SCVs showed prolonged persistence and decreased virulence, indicating diversification concerning their ecological lifestyle. This study underpins the importance of careful and tailored antibiotic treatments of infections with *B. cereus*, as therapy failure could possible induce persistent subpopulations. It also highlights the risk of misdiagnosis due to altered characteristics of the phenotype and metabolism of this *B. cereus* life form

Furthermore, this work also opens new questions regarding the ecological meaning of SCV subpopulation emergence and importance of SCV in spore former populations as an alternative route, next to sporulation, to cope with stress and adapt to specific ecological niches.

POSTER 14

Quantitative PCR tools for cultivation-independent analysis of microbial faecal pollution in water - the need for a continuous sample processing control

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PCR based techniques for the detection and tracking of faecal pollution have gained considerable popularity. One big advantage of using PCR based techniques over traditional culture of standard faecal indicator bacteria is that it directly targets the DNA present in a sample. The repeatedly observed phenomenon of organisms being viable but non-culturable (vbnc) can be overcome by targeting the DNA directly. However, applying molecular diagnostics to environmental matrices can be challenging, e.g. the PCR reaction can be inhibited and thus strongly biased by compounds such as humic acids or heavy metals. The aim of this study was a detailed process evaluation of the cell filtration, DNA-extraction and qPCR-performance characteristics applied to water samples from one habitat with a very diverse and changing matrix composition. The aquatic habitat selected was Lake Neusiedl located in the Eastern part of Austria, an important recreation area for the large urban area of Vienna. High and fluctuating levels of salt, humic acid, and suspended solids (TSS) characterize the water matrix of Lake Neusiedl. Therefore, this habitat represents a perfect "realistic world" model to study the basic performance characteristics of PCR-based diagnostics on water samples with changing matrix conditions. A filtration/extraction process control was added to each sample by spiking with a defined cell standard (DeToCS). In addition, a strict evaluation of PCR-inhibition by using an internal amplification control and different dilutions for each sample was performed. DNA was extracted using a phenol-chloroform and CTAB based method. Results clearly demonstrated that inhibition of the PCR reaction played only a minor role in (some) of the samples investigated. In contrast, the presence of high amounts of (inorganic) TSS led to a complete loss of DNA during the process of DNA-extraction (i.e. recovery rates ≤1%). To test the hypothesis that the adsorption of DNA to surface-reactive matrix components during DNA-extraction was the causing mechanism, experiments with different amounts of sodium pyrophosphate and salmon sperm were conducted in the laboratory. Addition of these adsorption site competitors helped to recover DNA concentrations to near control levels. The results of the presented study impressively show the importance of using a stringent and continuous process control on a "sample-per-sample" basis. Using this approach we were able to unmask the challenges of diverse matrices of water samples within a single habitat and could even adapt the DNA-extraction protocol in order to get comparable results. We postulate that the herein observed challenges are rather the rule than the exception. We think that this situation is conferrable to many surface waters, as high amounts of TSS are frequently observed (e.g. during event situation). We thus propose the continuous use of a stringent filtration/extraction process control as basis for a robust quality management of qPCR-based diagnostics for water quality monitoring. The use of such molecular based diagnostic tools will also gain importance in the future due to its potential to overcome the organism's state of being viable but not culturable. However, qPCR detects also dead cells. Future qPCR applications will thus also evaluate the possibility to apply viability stains to discriminate between live vs. dead cells in PCR analysis.

POSTER 15

Direct qPCR detection of human-associated *Bacteroidetes* MST markers in communal and domestic wastewater and comparison to cultivation-based standard indicators and human viral MST markers of faecal pollution

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Standard fecal indicators (SFIB) are determined by cultivation based techniques. Viable but non-culturable bacteria (VBNC) are not detected by such traditional water testing methods which can be a disadvantage for certain monitoring applications. A possible alternative is direct qPCR which detects DNA and hence also VBNC. Human-associated microbial source tracking markers based on intestinal *Bacteroidetes* populations (hB-MST) are increasingly applied to trace fecal pollution from communal and domestic sewage in water resources. HB-MST are often extending traditional monitoring schemes using SFIB. Considerable effort has been dedicated to fecal sensitivity and specificity testing of hB-MST assays during recent years. Emphasis has also been put on the efficacy of sampling techniques, DNA extraction, and PCR quantification. Until recently sound information on the occurrence of hB-MST in wastewater regarding the characteristics of the disposal system (size and type of the system), its seasonal variability and relationship to SFIB/alternative indicators (e.g. human-specific JC polyomaviruses, adenoviruses) did not exist. This study presents a detailed investigation of the seasonal occurrence, dynamics, removal and resistance of human-associated genetic *Bacteroidetes* fecal markers compared with ISO-based SFIB, human-specific viral MST markers and one human-associated *Bacteroidetes* phage in raw and treated wastewater of municipal and domestic origin. To recover representative results 24-hours integrated auto-sampling procedures at WWTPs were applied and multiplicative standard deviation (s*) was introduced to obtain an appropriate measure of statistical variability for log-normal distributed parameters. Characteristics of five representative municipal wastewater treatment plants (WWTPs) using mechanical and activated sludge treatment and from eight domestic WWTP from Austria and Germany were studied in detail (connected populations from 3 individuals to 49,000). hB-MST were consistently detected in high concentrations in raw (median $\log_{10}$ 8.6 marker equivalents (ME) 100 ml-1) and biologically treated wastewater samples (median $\log_{10}$ 6.2-6.5 ME 100 ml-1), irrespective of plant size, type and time of the season (n=53-65). HB-MST and SFIB concentrations revealed the same range of statistical variability for raw (s*=2.3-3.0) and treated wastewater (s*=3.7-4.5), with increased variability after treatment. In raw wastewater correlations among microbiological parameters were only detectable between hB-MST, *C. perfringens* and JC polyomavirus. Statistical associations amongst microbial parameters increased during wastewater treatment. Two plants with advanced treatment were also investigated, revealing a median log10 4 reduction of hB-MST in the activated sludge membrane bioreactor, but no reduction of the genetic markers during UV irradiation (254nm). In conclusion, this study highlights the huge potential of human-associated HB-MST to complement wastewater impact monitoring based on the determination of SFIB for recent fecal pollution events. In addition, human-specific JC polyomavirus and human adenoviruses seem to be a very attractive support if highly specific and more persistent markers are needed to complement the monitoring approach. HB-MST detected by qPCR overcome the VBNC challenge for bacteria as far as they correlate with cultured SFIB. However, HB-MST also detects dead bacterial cells. Future qPCR applications will also evaluate the possibility to apply viability stains to discriminate between live vs. dead cells.

POSTER 16

Persistence of planktonic Pseudomonas aeruginosa under swimming pool conditions

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Pseudomonas aeruginosa is a Gram-negative dweller of moist habitats, as well as an important opportunistic human pathogen. Diverse national regulations on swimming pool maintenance mention it as a process indicator. Consequently, in the according countries, swimming pool water needs to be controlled for and kept free of *P. aeruginosa*. Detection usually is accomplished via culturing methods.

One common agent used for residual disinfection of swimming pool water is hypochlorous acid (HClO). Experiments were performed investigating disinfection kinetics of this agent with planktonic inocula of several bacterial species. A setup mimicking the conditions in swimming pool basins was designed for this purpose. Kinetics for the disinfection of *P. aeruginosa* were within a range covered in German regulations.

However, results also indicated that inactivation of *P. aeruginosa* was not a terminal process in every case. The question arose whether the applied culturing methods were sufficient for displaying the decline of bacteria properly. Recovery experiments revealed the option of resuscitation after colony counts dropped to zero during disinfection. Furthermore, exposure to hypochlorous acid in some cases led to the occurrence of an altered colony morphology (smaller, lacking coloration).

It is hypothesized that *P. aeruginosa* may survive swimming pool conditions without being traceable with standard culturing methods. Current investigations focus on proving this true as well as on illuminating underlying mechanisms.

POSTER 17

Effect of adverse conditions of the gastrointestinal tract on lactic acid bacteria from milk products of functional nourishment

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Among the probiotic correctors of normal microbiota the special interest present the lactic acid bacteria (LAB) isolated from national lactic acid products of functional nourishment. The Buryat (Mongolian) Kuruna and Lebanese products Laban and Laben used to treat a number of diseases of the gastrointestinal tract (GIT) and cardiovascular system for several thousands of years. We isolated some strains of LAB with high antagonistic activity against pathogenic and opportunistic bacteria. The lactococci strain from kurunga was identified as *Lactococcus lactis* ssp.*lactis* K-205 (GenBank EF 114305) and two of lactobacilli strains were *L.diolivorans* KL-1 and KL- 2 (JF 520627, JF 520433), also *L.delbrueckii* ssp. *bulgaricus* 2.5 MSU (from Laban), *L.paracasei* 9-MSU (from Leben). Growth of the indigenous microbiota in the macroorganism depends on the processes in bacteria, including free radicals. Reactive oxygen species trigger programmed cell death – apoptosis. Strong oxidative stress indicates that the cells have antioxidant defense enzymes, such as superoxide dismutase (SOD). The goal of this work was to study of effect of adverse conditions of GIT on effective strains isolated from Kurunga and Lebanese products (Laban and Leben). In the model experiments the strains were resistant to the action of bili acids: with the concentration of 0.8 to 1.0 % its viability comprised 92.0 % (1.31×10^9 – 9.1×10^8). Their survival at 0.6% HCl for 3 hour exposition was 9.1×10^8 – 6.8×10^7. The strain *L.paracasei* 9 was more resistant to HCl (1.25×10^9 to 0.98×10^9). It was found that strain K-205 had a relatively high SOD activity (25.74 U/mg of protein) but *L.diolivorans* KL-1 had only 5.9 U/mg. The unique properties of isolated novel strains such as wide spectrum of activity against pathogens, stability under conditions of GIT, relatively, high SOD activity as the fight against serious diseases and early signs of aging allow to recommend them for creating probiotic cultures.

POSTER 18

Defining the relationship between antibiotic persisters and the viable but nonculturable state: physiologically related phenotypic variants on the continuum of dormancy

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Viable but nonculturable (VBNC) cells and antibiotic persisters are well-described phenotypic variants that make up dormant subpopulations allowing bacteria to survive in conditions that are deadly to the rest of their genetically identical lineage. There is growing evidence that these forms of dormancy share molecular mechanisms that govern their growth dynamics and that these dormant forms are co-occurring. However, the current literature portrays them as distinct entities with strict definitions, and only rarely mentions them together. Here we describe what has come to be known as the dormancy continuum hypothesis, the notion that heterogeneous populations are distributed along a range of varying levels of dormancy, with rapidly growing cells and VBNC cells nearing the extremes of the continuum. Here we first illustrate the evidence supporting a shared molecular mechanism that governs these phenotypes, then discuss recent independent studies that experimentally support the dormancy continuum hypothesis. Finally, we evaluate the impact of recognizing the relationship between these two phenotypes as it relates to infectious disease research and the development of novel therapeutics.

POSTER 19

Bacteria lifecycle in the FMCG sector

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Knowing of bacteria lifecycle is essential in the FMCG sector, as most products can be contaminated by them. The article identifies few bottlenecks and investigates the ideal de-contamination procedure related to these cases, which ends up a higher manufacturing culture and lower object security costs. To identify the risks, methods of statements can be borrowed from the medical researchers, as they have good know-how of low contaminated areas such like theaters. Any new method of work is pulled into this sector can end up huge cost, so this article investigates some real risks within the supply chain. The case study is based on a manufacturing environment, where the product is sliced cold meat. This product can be contaminated before- during- and even after the manufacturing process which can end up in poor quality and health risks. The methodology of the research is a comparative analysis. As in the medical industry most of the technics are already used, we can have the question how to adopt these procedures to the FMCG sector. We can have the question how to estimate and manage the costs in case if any bottleneck had been already investigated.

POSTER 20

Extended spectrum beta lactamases (ESBL) harboring Escherichia coli from different sources, dealing with nutrition restrictions and different growth temperature

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Introduction

Spread of human induced antibiotic resistance outside the clinics is an alarming and not fully understood phenomenon. Especially *Enterobacteriaceae* with their prominent member *Escherichia coli* seem to reveal as resistant strains everywhere. Conditions for growth and survival in surface water are quite different to the natural habitats of *E. coli* and they bear the additional burden of resistance genes.

Aim

ESBL *E. coli* isolates from clinical, water and sediment samples were analyzed on their ability to deal with different growth temperature and nutrition media.

Methods

Growth (OD600) of 23 ESBL *E. coli* isolates and seven *E. coli* isolates without any resistance from different origins was measured for 24h. Growth curves at different temperatures and with different nutrient media (LB, M9) were performed.

Results

No difference in growth between the tested *E. coli* strains could be observed In LB full media at 37°C. The log- phase started in all strains at similar time points and showed same characteristics. At 20°C the strains showed differences in their growth- behavior. Highest cell mass was achieved by a ST940 river isolate with an OD600 of 1,287. Lowest values were reached by a ST131 river isolate (from clinical origin) with an OD600 of 0,751 after 24h. In minimal media (M9) the growth behavior was more diverse at 37°C and at 20°C, even at early time points. Highest amounts at 37°C with M9 media could be shown in a ST154 river sample with an OD600 of 0,386 after 24h. Lowest value was reached by samples without any resistance.

Conclusion

The combination of low nutrients and low temperature seems to give a positive advantage for the river samples. Within this study, we saw that *E. coli* river isolates are better adapted to stress conditions, especially under low temperatures and low nutrient conditions, compared to isolates from clinical origin.

POSTER 21

Agar matters: Cultural detection of the pathogen Burkholderia pseudomallei and other *Burkholderia* species

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The ß-proteobacterium *Burkholderia pseudomallei* is the causing agent of the neglected tropical disease melioidosis. This disease is endemic in, but not restricted to Southeast Asia. It was estimated that 165,000 cases of human melioidosis occur worldwide per year, from which 89,000 people die. Melioidosis could be acquired through inoculation or aerosols of *B. pseudomallei* from contaminated environments such as soil and surface waters. Unfortunately many regions exist where melioidosis did occur but *B. pseudomallei* could not be isolated from the environment. This indicates amongst others the ineffectiveness of recommended culture media to promote growth of dormant/starving *B. pseudomallei* from soil, which might lead to false negative culture results due to a so called "nutrient shock". Here, we focused on the effect of growth agar composition/nutrient availability on *B. pseudomallei* and other *Burkholderia* precultured under low nutrient conditions. As an example, we compared the tryptic soy agar (plus selective agents) based *B. pseudomallei* agar (Ashdown´s Agar) to R2A-Agar using pure cultures and an artificial soil approach. We could show that *B. pseudomallei* and the near neighbor-species *B. thailandensis* were differently affected on Ashdown´s agar or R2A. Moreover, the usage of R2A lead to slightly increased recovery rate of *B. pseudomallei* in artificial soil microcosms compared to Tryptic soy agar and Ashdown´s agar. These findings suggest that a re-definition or refinement of culture media could lead to improved recovery and detection rate of *B. pseudomallei* from soil or other habitats with low nutrient availability. Moreover the presented experimental models might help to understand the eco-physiology of *B. pseudomallei* in the future.

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Staphylococcus aureus strains under CuCl2 or CuSO4 influence - Impedance analyses of biofilm formation as a new analytical tool

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The preferred form of growth of bacteria in nature is biofilm formation. Biofilms often lead to significant problems in clinical settings as they are hart to remove from surfaces (e.g. catheters) and the show increased antimicrobial resistances due to physical protection of the cells and due to the lower exchange rate of the cells with their environment. Staphylococcus aureus (strain DSM-799 and Newman) biofilms were grown for 48h in different concentrations of CuCl2 or CuSO4 in order to establish a copper value giving a breakpoint for cell growth. Electrical impedance sensing (EIS) was performed using an ECIS Model Z Theta (Ibidi, Germany). Biofilms were grown in ECIS culture ware eight well arrays with ten electrodes per well optimized for adhesion studies and other cellular assays. Starting value impedance was measured using 150 µL of LB media and 150µL of the copper solution in the desired concentration. This step was done for all eight cells and the solution was carefully removed with a pipette after the measurement. Afterwards, again 150µL of the desired copper concentration were added to the empty cells and inoculated with 150µL of the diluted ONC (OD600 of 0.5). The impedance plates were sealed using breathable membranes. Impedance was measured directly afterwards and after 24 and 48 hours of incubation at 37°C and 90 rpm. Additionally typical laboratory measurements for biofilms (polysaccharide and protein content, life/dead cell flow cytometry) were performed. When uninfluenced, biofilms develop on the electrodes of the system, blocking the electrodes, causing the impedance values to drop. When cell growth and biofilm formation becomes inhibited this drop does not take place. These findings are supported by the data of the other measurement techniques. Future studies will evaluate the feasibility of the presented method for use in routine antimicrobial assessment. Other species and different antimicrobial substances will be used.

NOTES

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