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ABSTRACTBOOK

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Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

345/EEP
Circum-neutral pH and Low Temperature Define *Candidatus* Nitrotoga spp. as Competitive Nitrite Oxidizer
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So far *Candidatus* Nitrotoga spp. is known as a cold adapted nitrite oxidizing bacterium (NOB), with enrichments originating from permafrost soil of the Siberian Arctic and a cold water recirculating aquaculture system (RAS). Apart from these habitats, *Nitrotoga*-like NOB could be detected in different natural and technical environments. They are thus of importance for the global nitrogen cycle and furthermore contribute to a successful nitrification in wastewater processing. However, *Nitrospira* spp. can grow under low temperature conditions as well and they often coexist with *Nitrotoga*-like bacteria. Hence the question of niche separation between these two NOB arises.
In this study, we focused on the influence of pH on distribution of *Nitrotoga* and *Nitrospira* in co-culture. A highly enriched *Nitrotoga* from the WWTP in Hamburg-Dradenau, *Ntg.* BS, was characterized regarding its pH and temperature optimum, and subsequently combined with *Nitrospira defluvii*. Co-cultivation of both NOB was set-up in two batch bioreactors at 17°C with different pH in parallel runs using 1 mM nitrite.
Ntg. BS clearly outcompeted *Nsp. defluvii* at pH 7.4 and 17°C, correlating with its pH optimum at 7.3. Since *Nsp. defluvii* has the same pH optimum, *Ntg.* BS was probably at an advantage due to the low temperature, as it grew optimal at 17°C. At pH 6.4, no distinct predominance of either NOB was observed. However, *Ntg.* BS was never suppressed, as was *Nsp* under more adverse conditions. Thus, we could demonstrate *Ntg.* BS as a competitive NOB, dominating over *Nsp. defluvii*, when environmental parameters allow optimal growth of this cold-adapted organism. These findings confirm the importance of *Nitrotoga* spp. for nitrification in cold environments and technical applications.

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346/EEP
Searching new extremophilic microbial model systems for space exploration studies – data from a large-scale transect study in the Atacama Desert
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The search for new model organisms for space exploration missions in the Atacama Desert is traditionally limited to a rather narrow strip (Yungay, Chile) which is believed to have the most arid conditions on Earth (McKay et al., 2003) thus harboring the most desiccation-resistant microorganisms. However, it is not clear whether Yungay is indeed the driest place in the Atacama, as this should be the one with the lowest soil organic carbon (SOC) stock and soil water (SW) content. Therefore we tested different soil samples from an aridity-gradient transect with comparable sites (inclination, position in the rain shadow of the coastal mountain range, approx. 100 km distance between the sites) spanning roughly 600 km in the Atacama Desert for SOC stocks and SW content. We found, that SOC stocks decreased with aridity from 25.5 to 2.1 kg m^{-2} cm^{-1}, while the SW contents decreased at 5 of our sites and increased in the hyper-arid zone. To our surprise, we identified one site located 100 km north of Yungay which had substantially lower SOC stocks (1.92 kg m^{-2} cm^{-1} ± 0.73) than Yungay (2.21 kg m^{-2} cm^{-1} ± 0.75), but with 0.043 g of water per 1 g of soil ± 0.03 comparable SW contents, while Yungay has 0.043 g ± 0.06. Thus we consider this site to display different growth conditions and ecological niches as compared to Yungay and therefore as promising candidate site for the identification of new species of radiation-resistant microorganisms, as the resistance against desiccation is paired with a distinct resistance to ionizing radiation due to efficient microbial DNA repair mechanisms (Mattimore et al., 1995). Soil samples were irradiated with high doses of gamma radiation up to 25 000 Gy. Surviving colonies were cultivated on a medium favoring the growth of *Deinococcus*-like species and their affiliation was determined using 16sRNA-Next Generation Sequencing. Here, we evaluate the hypothesis of ecological niching even at the most hyper-arid places of our planet on grounds of our recently identified site – with implications for the search for life in hyper-arid Martian regolith in future robotic space exploration missions such as ExoMars.

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347/EEP
Survival of the NASA Mars Odyssey isolate *Acinetobacter radioresistens* 50v1 on different spaceflight relevant antimicrobial surfaces
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Since many years, human mankind travels to space. One of our mayor interests is the health of astronauts and the protection of the spacecraft. Apart from external influences, the microbial burden inside of the International Space Station (ISS) may be dangerous and must be limited to a minimum. To ensure the status and the protection of the crew as well as the spacecraft itself, it is necessary to determine the survival of microorganisms on different surfaces. Microorganisms are constantly changing their strategy of survival, primarily induced by extreme environmental conditions, such as space conditions, compared to their terrestrial habitats. However, the increased levels in resistance and robustness possibly play a sensitive role in evolving new virulence factors in the space environment.
One of the bacteria on the NASA Mars Odyssey spacecraft, which have been isolated, is the Gram-negative, non-motile bacterium *Acinetobacter radioresistens*. Apart from *Deinococcus radiodurans*, *A. radioresistens* shows similar levels in radiation and oxidative stress tolerance (McCoy et al., 2012). In our work,

we used the strain 50v1, isolated from the surface of the Mars Odyssey spacecraft as well as the type strain DSM6976, which was isolated on Earth from cotton and soil samples. We investigated the resistance regarding in their desiccation tolerance on metallic surfaces including materials with different antimicrobial properties. For those experiments we exposed and desiccated both strains on the different surfaces (such as copper- and silver-containing materials) and determined the survival over different time points. First results show a high resistance of the spacecraft isolated strain compared to the type strain. These results give implications about the higher survivability of environmental microorganisms and highlight the essence of bioburden reduction and improve sterilization approaches/techniques for upcoming space exploration missions towards the search for life outside Earth.

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348/EEP

Peptostreptococcaceae and _Aeromonadaceae_: Drivers of protein- and RNA-based fermentation in gut contents of the earthworm _Lumbricus terrestris_

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By virtue of their feeding habits, earthworms are soil engineers of the terrestrial biosphere. Ingested soil-derived fermenters are conceived to be activated by the favorable conditions (e.g., anoxia and high concentrations of worm mucus-derived saccharides) in the gut of earthworms. These activated fermenters could theoretically drive the degradation of biopolymers derived from disrupted ingested plant and microbial biomass, and thus provide organic acids that could be utilized by the earthworm. The objective of this study was to resolve the capacity and identity of gut microbiota potentially linked to the degradation of biopolymers during gut passage. Anoxic microcosms of gut content of the model earthworm *Lumbricus terrestris* were supplemented with the biopolymers cellulose, xylan, protein, and RNA. Fermentation (i.e., the production of CO_2, H_2, and organic acids) was strongly stimulated in protein and RNA treatments. In contrast, fermentation was only minimally stimulated by cellulose and xylan. These results indicated that protein and RNA, rather than cellulose and xylan, are subject to rapid degradation by gut microbiota. Ilumina-based 16S rRNA and 16S rRNA gene sequencing was utilized to identify microbes potentially linked to the degradation of protein and RNA. These analyses indicated that the *Peptostreptococcaceae, Clostridiaceae*, and *Fusobacteriaceae* were primarily linked to the degradation of protein, whereas the *Aeromonadaceae* were primarily linked to the degradation of RNA. The differential stimulation of fermentative taxa with protein and RNA suggests that the engagement of biopolymer-linked fermenters in the gut is biopolymer-specific. The collective data indicated that protein and RNA, the two main soluble biopolymers released via the disruption of microbial cells in the gizzard, are subject to hydrolysis and fermentation by microbes in the alimentary canal. Thus, gut-associated fermentation of protein and RNA likely (a) contributes to the fermentation dynamics in the alimentary canal, and (b) yields important sources of organic carbon (i.e., organic acids) for both the catabolism and anabolism of the earthworm.

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349/EEP

A high-throughput-approach for the cultivation of bacterial consortia from eukaryotic hosts including a screening method for new antimicrobial compounds

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A large number of bacteria and fungi have the ability to produce a variety of secondary metabolites. Some of these Natural Products are of high importance for the use in the pharmaceutical industry e.g. as antibiotics. Due to the increasing occurrence of resistant bacteria there is a high demand for the discovery of new antimicrobial compounds.

Symbiotic bacterial communities interact among each other and with eukaryotic hosts by the production of a broad range of secondary metabolites and quorum sensing molecules. Secondary metabolites often play here important roles by driving the composition of symbiotic microbial communities and protect both, the host and the host-specific microbial consortia, for pathogen invasion. For this reason, a promising strategy to find new antibiotics is the enrichment of actively interacting (metabolite producing) bacterial consortia from highly competitive habitats and screen those enrichment cultures for their antimicrobial activities.

The aim of our study was the enrichment of bacterial assemblages from natural habitats in a 96-well plate based dilution-to-extinction cultivation approach, where slow-growing bacteria are protected from overgrowth. Furthermore, not-yet-cultured or so far unculturable bacteria may be enriched in consortia in case of a co-enrichment of the special interaction partner in the microtiter plate well. In one parallel workflow, enrichment cultures were preserved for long-term storage, DNA was extracted for further analysis and a pre-screening for the production of secondary metabolites (antimicrobial compounds) and quorum sensing molecules was performed using specifically established spot-assays. Antimicrobial active enrichment cultures were differentiated at the strain level by genomic fingerprinting and phylogenetically identified by 16S rRNA gene sequencing. The results of the pre-screening and molecular identification are the bases for further tests regarding the bioactivity of the pure cultures and the bacterial consortia.

To establish this cultivation strategy, we tested both bacterial symbionts of marine corals and sponges, which are well-characterized holobionts and endophytic bacteria from rapeseed root and hypocotyl. First results indicate that the strongest inhibition of pathogenic test strains seem to appear in the presence of a co-culture with more than one strain, e.g. found for a *Pseudomonas* co-culture. Currently we investigate in more detail if single strains or only co-cultures lead to the antimicrobial activities.

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Figure 1: 3D-Structure of Lcp_{K30} and myoglobin in a 3-3 globin fold:

Figure 1

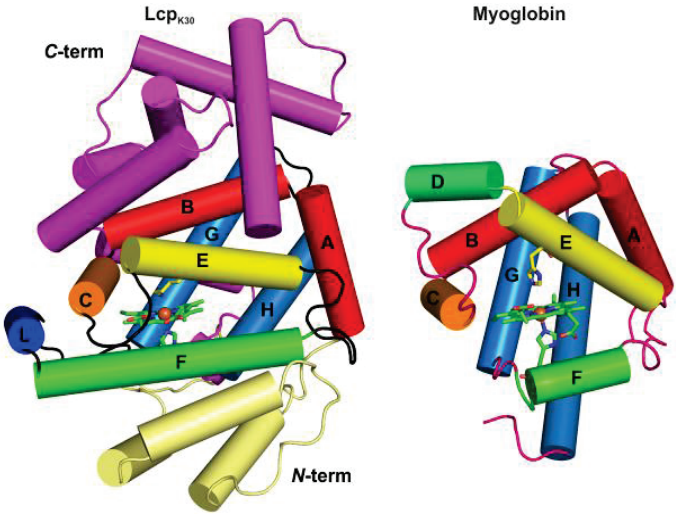
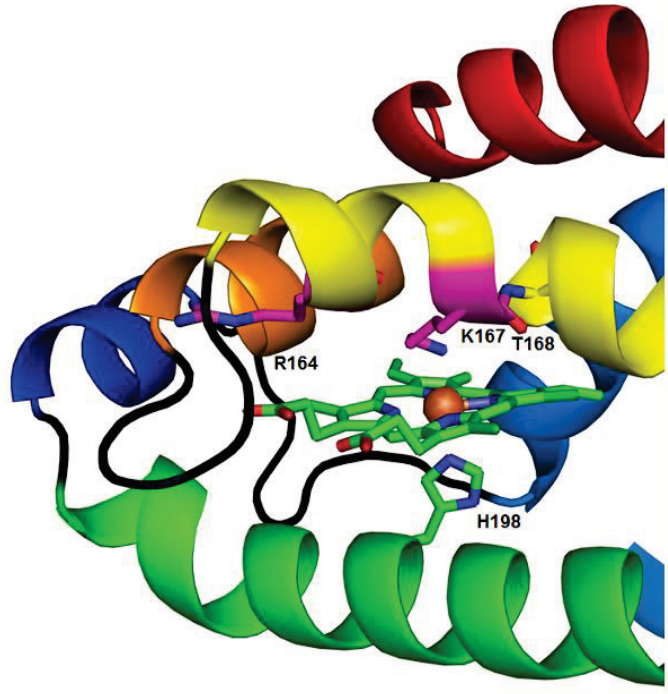


Figure 2: Active site of Lcp_{K30}:
Figure 2



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382/EEP
Translating physics to microbiology: spore resistance to terrestrial and extraterrestrial extremes

R. Moeller*[1]
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Spore-forming bacteria are of particular concern in the context of planetary protection because their tough endospores are capable of withstanding certain sterilization procedures as well as harsh environments (Nagler et al., 2015, 2016; Nicholson et al., 2012). Spores of *Bacillus subtilis* have been shown to be suitable dosimeters for probing extreme terrestrial and extraterrestrial environmental conditions in astrobiological and environmental studies. During dormancy spores are metabolically inactive; thus substantial DNA, protein, tRNA and ribosome damage can accumulate while the spores are incapable of repairing and/or degrading damaged DNA and proteins. Consequently, damage to essential components of spores poses a unique problem, since damage repair does not occur until the processes of spore revival. Spores appear to have two possible ways to minimize deleterious effects of environmental extremes: (i) by protecting dormant spore macromolecules (in particular the spore DNA) from damage in the first place and (ii) by ensuring repair of damage during spore outgrowth. In our research, we used spores of different genotypes of *B. subtilis* to study the effects of various extraterrestrial conditions (e.g., planetary conditions as present on Mars or low Earth orbit (LEO)) for astrobiological purposes. Spores of wild-type and mutant *B. subtilis* strains lacking various structural components were exposed to simulated Martian atmospheric, galactic cosmic and UV irradiation conditions. Spore survival was strongly dependent on the functionality of all of the structural components, with small acid-soluble spore proteins, coat layers, and dipicolinic acid (DPA) as key protectants. In addition, the interaction of several DNA repair mechanisms (e.g., non-homologous end joining (NHEJ) and spore photoproduct (SP) lyase) was identified as crucial for surviving environmental extremes in space or Martian surface (i.e., exposure to solar UV and galactic cosmic radiation (Moeller et al., 2012). The ultimate goal is to obtain a complete model describing spore persistence and longevity in harsh environments.

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CLIP peaks highlighted a consensus motif, CUG, as a common feature in the CspC and CspE binding regions.
To obtain a detailed molecular mechanistic view of CspC and CspE function, we used *ecnB* mRNA as a model transcript. It contains two CLIP-predicted common binding sites and it is negatively regulated in the double *cspCE* deletion strain. We found that the expression of the *ecnB* mRNA depends on the presence of CspC or CspE, which bind and stabilize the transcript post-transcriptionally. To dissect the molecular determinants of Csp-mediated regulation of the *ecnB* mRNA, we used *in vitro* gel shift assays with different mutants of the predicted binding sites. Only one of the two CLIP peaks of *ecnB* is essential for binding of CspC and CspE. Moreover, a single point mutation in the CUG motif inside this peak decreased significantly the affinity to the proteins, validating the *in silico* prediction. Computation of *ecnB* mRNA folding revealed that the second peak is involved in the formation of a stem-loop, suggesting that both sequence and structural elements could indeed be required for binding to CspC and CspE.

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POSTERSESSION
General and Hospital Hygiene (StAG HY)

549/HYP
Understanding the molecular mechanisms involved in the spore inactivation by plasma sterilization

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Being the most resistant form of a biological system, spores of *Bacillus subtilis* are very resistant against a broad spectrum of sterilization methods and, therefore, are commonly used as a biological indicator in order to verify the functionality of a sterilization procedure. The process of low-pressure plasma sterilization is a promising alternative to conventional sterilization methods as it is extremely fast, efficient and gentle to heat-sensitive materials. Active plasma species contain a high degree of sporicidal UV/VUV-radiation, as well as charged particles and free radicals, which exert detrimental effects on microorganisms. In this study we present novel insights into the key factors involved in spore inactivation by low pressure plasma sterilization using a double inductively-coupled plasma reactor.
In order to standardize the assessment of spore inactivation efficiencies by plasma discharges, an electrically operated deposition device was developed, allowing fast, reproducible, and homogeneous preparation of *B. subtilis* spore in monolayers on surfaces leading to more reliable investigations. We demonstrate that low-pressure plasma discharges of argon and oxygen discharges cause significant physical damage to spore surface structures as visualized by atomic force microscopy. A systematic analysis of *B. subtilis* spores lacking individual coat and crust layers - the first barrier to environmental influences – revealed the coat to be a major factor in spore resistance towards plasma treatment (Raguse et al., 2016).
In order to gain a better understanding of the complex molecular mechanisms involved in the inactivation by plasma sterilization processes, we analyzed plasma-induced DNA lesions in vitro, identified general and spore-specific DNA lesions, and characterized different DNA repair mechanisms during spore revival after plasma treatment.

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550/HYP
Development of a site-specific bioluminescence-based test system for *in vivo* evaluation of antimicrobial coatings

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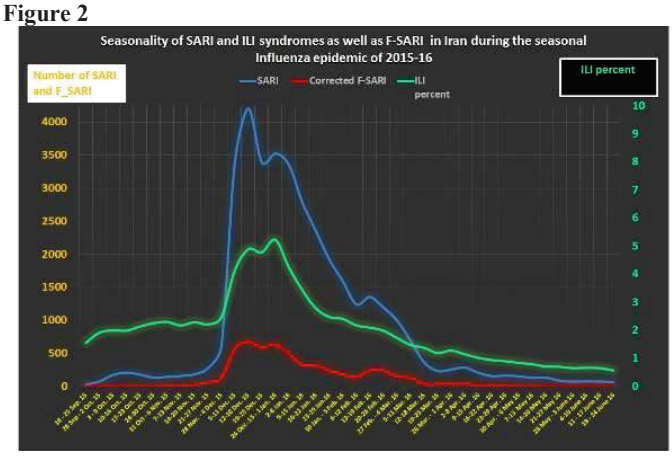
In almost every technical system, which is in contact with aqueous liquids, bacterial biofilm formation occurs and may cause hygienic problems. Antimicrobial coatings of surfaces are a feasible approach for minimizing biofilm formation and, thus, for increasing the safety and performance in such technical installations.
For establishing novel antimicrobial coatings it is necessary to evaluate the efficiency of the new material. The Japanese Industrial Standard JIS Z 2801/ ISO 22196 assay is routinely used for this purpose. However, this test does not reflect the biotic and abiotic conditions prevailing in many technical water installations, e.g. in drinking water filtration where the concentration of organic nutrients is extremely low.
Thus, the goal of this study is to develop a site-specific test system for antimicrobial coatings for ultrafiltration membranes, which is based on bacteria that actually occur in the respective sites of application. As bioluminescence is a well-established indicator of bacterial fitness and allows *in vivo* analysis of antibacterial effects, the respective test bacteria will be equipped with the *lux*-operon.
Preliminary work with a bioluminescent strain of *Escherichia coli* MG1655 showed that bioluminescence can be detected inside the fibers of ultrafiltration membranes and that the respective antimicrobial compounds designated for the use in the coatings interfere with the cells ability to perform bioluminescence. In the next step, bacteria were isolated from commercial membranes for drinking water ultrafiltration and identified as *Alphaproteobacteria* of the genera *Sphingomonas* and *Novosphingobium*. These genera are known to occur in anthropogenic as well as in oligotrophic natural environments. The isolated bacterial strains are currently being characterized regarding substrate spectra, biofilm formation as well as resistance and survival of stress conditions, especially starvation. Selected strains will then be engineered as bioluminescent reporter strains and used for in vivo analysis of biofilm formation in coated and non-coated membrane fibers.
The approach pursued in this study has the potential to evaluate the performance of antimicrobial coatings with site-specific bacterial strains under conditions close to the *in-situ* situation.

Figure 2



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

807/MSP

Estimation of influenza and severe acute respiratory illness incidence (burden) in three provinces of the Islamic Republic of Iran, 2012 and 2013

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Question: A significant proportion of the global burden of acute lower respiratory infections is attributable to influenza and respiratory syncytial virus. There are few estimates of influenza burden in the World Health Organization Region for the Eastern Mediterranean. In this study we estimated the burden of severe acute respiratory infection (SARI) and flu-associated SARI (F-SARI) in selected provinces of Iran, the trends of SARI and confirmed cases of influenza (F-SARI) in 12 months (seasonality) and the age groups most at risk of SARI and F-SARI.
Methods: Using the electronic Iranian influenza surveillance system and data of cases in sentinel hospitals of 3 selected provinces, we estimated the monthly trend (seasonality) of incidence for SARI and F-SARI, overall incidence of SARI and F-SARI and their disaggregation by age with the aid using the Monte Carlo technique.
Results: The incidences for SARI and F-SARI for all age groups was 187.6 and 29.0 per 100 000 population, respectively.
Conclusions: A seasonal pattern in epidemics of influenza and SARI was observed similar to other countries of the northern hemisphere with several peaks in cold months. The age groups most at-risk were children aged under 2 years and adults older than 50 years.

Figure 1

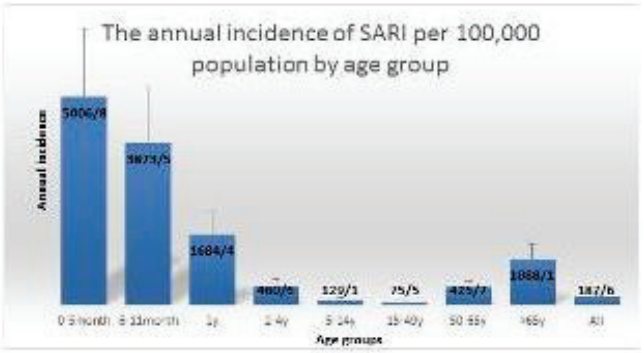
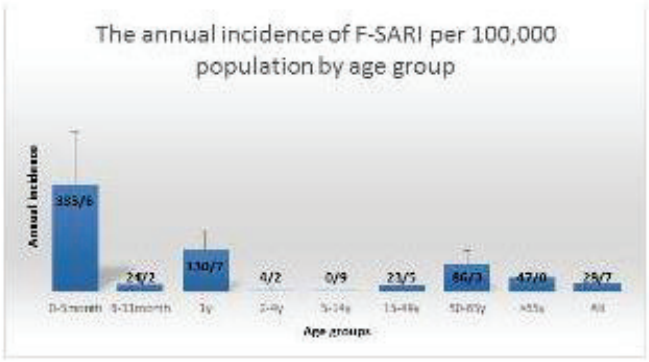


Figure 2



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808/MSP

Influence of simulated microgravity on *B. subtilis* biofilms

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Bacillus subtilis is one of the most studied Gram positive model organisms. Since mission Apollo 16, *B. subtilis* has been used for a multitude of space experiments. Investigating the influence of extreme conditions like those in space, non-domesticated strains, such as NCIB 3610 are of special interest regarding their ability to form biofilms. Since it is known that planktonic life is the exception, biofilms are considered as predominant way of living (Moons et al., 2009). Biofilms are organized in a complex self-produced extracellular polymeric matrix commonly composed of polysaccharides, proteins and nucleic acids. Building a biofilm protects the individual cell against shear forces, chemicals (e.g. antibiotics or disinfectants), temperature changes and water as well as nutrient depletion (Vlamakis et al., 2013, Cairns et al., 2014). The intrinsic resistance of biofilms is a problem, not only in industry and medicine, but it can be problematic under spaceflight conditions. Especially the loss of gravity coupled with changed levels of radiation might influence the resistance and therefore the virulence of bacterial biofilms. This can possibly evoke problems for the crew as well as for the spacecraft. In particular, long term missions with complex cooling systems, water supply and heat pipes may be vulnerable to biofilm colonisation.

In our work, we used the biofilm-forming wildtype strain NCIB 3610 and a biofilm-matrix deficient mutant (deletion of 15-gene exopolysaccharide operon, epsA-O) to study the impact of reduced gravity on maturated biofilms. Our major research goal is to compare biofilm formation in simulated microgravity (using a 2D clinostat) to terrestrial gravity (1g) conditions by using different microscopic techniques. White light profilometry, scanning and transmission electron microscopy (SEM, TEM) and confocal laser scanning microscopy (CLSM) were used to analyse biofilms regarding their topology and inner structure, respectively. First results show qualitative architectural differences between simulated microgravity and 1g in cross-sections, but no significant qualitative variations in biofilm surface topography.

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809/MSP

Development and evaluation of a novel vaccine against neoteric serotypes of *Streptococcus pneumoniae* prevalent in Egypt

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Introduction: *Streptococcus pneumoniae* is still one of the major causes of morbidity and mortality worldwide especially among infants. The prevalent serotype distribution shows variation at different time intervals. In order to assess efficiently the epidemiology of the diseases for effective preventive and treatment strategies, serotype prevalence need to be periodically reassessed for the formulation of effective vaccines**.**
Objectives: The aim of this work is to determine the most recent serotypes of *Streptococcus pneumoniae* prevalent in Egypt, to prepare the conjugated capsular polysaccharide vaccine from these particularly predominant serotypes and evaluate them in vivo in an animal model.
Materials and Methods: Clinical specimens representing different cases of streptococcus infections were collected from the Greater Cairo area in Egypt. Conventional and molecular identification methods were performed, the antimicrobial susceptibility patterns were assessed and serotyping was done using PCR to identify the most prevalent serotypes. Capsular polysaccharides from the most current and prevalent serotypes were extracted, purified and conjugated to bovine serum albumin. The polysaccharide protein conjugates were purified through ultrafiltration technique and the molecular size distribution was determined compared to an available vaccine. The Immunogenicity of the prepared vaccine was examined in vivo by two different methods. First by measuring the elicited antibodies levels in blood after mice vaccination. Second by challenging the vaccinated mice groups with each serotype and determining the degree of protection offered by the developed vaccine.
Results: The results showed that among the clinical specimens collected, serotypes 6A/B and 19F were the most predominant. An alarming rise in antibiotics resistance among different isolates was observed. The conjugated capsular polysaccharide vaccine prepared from both serotypes revealed significant immunogenic effect in both in vivo methods examined. The vaccines prepared induced a rise in antibody levels as measured by Enzyme-linked immunosorbent assay (ELISA) and were able to increase the survival rate of the mice challenged with *Streptococcus pneumoniae* compared to appropriate animal control groups.
Conclusion: It is essential to track the most recent and prevalent serotypes of *Streptococcus pneumoniae* to prepare relevant, efficient and cost-effective vaccines particularly in developing countries.

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POSTERSESSION
Phage and CRISPR (FG PC)

810/PCP

A novel phage phiE72: an alternative therapeutic against *Staphylococcus epidermidis* infection and a potential research tool

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Staphylococcus epidermidis is one of the most common pathogens causing various types of nosocomial infections in hospitals, mainly by forming biofilms on medical devices. Nowadays, the situation of increasing number of *S.epidermidis* developed resistance to antibiotics is calling for alternative therapeutics. Besides, a novel research tool is also expected since study of the pathogenicity of *S.epidermidis* is limited due to genetic manipulation failure caused by strong genetic barrier mechanisms, especially the clinical ones. Recently, we isolated a new bacteriophage named phiE72 from a *S.epidermidis* strain in an infected tooth of a clinical patient. Electron microscopy revealed characteristics as bacteriophages of the *Siphoviridae* family. Phage infection assay using different bacterial species showed that phiE72 has a narrow host range and is specific to *S.epidermidis*. It showed a more drastical decrease of turbidity of bacterial host cell culture even compared to the widely studied antibiotic reagent member lytic polyvalent phage phiK. PhiE72 remained stable at pH values between 5.0 and 8.0 and up to the temperature of 60 °C . PhiE72 also showed tolerance to chloroform. The fast and strong lyse property, and specificity for *S.epidermidis* indicates the novel phage phiE72 an attractive candidate for phage therapy or as a biofilm eradication agent against *S.epidermidis*. Moreover, phiE72 can transduce plasmid DNA efficiently even to strains refractory to electroporation. Therefore, phiE72 might also become a valuable research tool for plasmid transduction for *S.epidermidis* strains, which are often difficult to transform.

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811/PCP

Isolation, characterisation and genomic analysis of bacteriophages against ESKAPE pathogens

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Introduction: The presence of multi-resistant bacteria, e.g. in the hospital environment, causing severe and life-threatening infections is a huge danger that urgently has to be overcome. The lack of new antibiotics against clinically relevant ESKAPE pathogens (*Enterobacter ssp.*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa, E. faecium*) calls for new medical approaches and agents to fight those. Bacteriophages are viruses that specifically infect and eliminate bacterial strains of one species and therefore might be an alternative to targetedly combat multi-resistant pathogens.
Objectives: This project aims to isolate and characterise bacteriophages against several multi-resistant ESKAPE pathogens, in particular *E. coli* and *S. aureus*, in order to evaluate

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