PS22 – Microbial Disease Ecology

351A Virulence and antibiotic resistance properties of *Stenotrophomonas maltophilia* linked to ecological niche adaptation
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Actual patterns of human activities affect in two ways the emergence of infectious diseases. In the clinical context, microbial communities are considerably impacted by antibiotic overuses for patient treatments. Many multiresistant strains have then emerged under these selective pressures, causing a major concern to human health. In the environmental context, anthropic disturbances on ecosystems lead to adaptations in the metabolism of microbial communities. Particularly in a heavy metal polluted environment, microbial communities can develop resistance mechanisms. These mechanisms were found to favour resistance against antibiotics during clinical treatments. Some clinical pathogens characterized as opportunistic pathogens can be isolated from an environmental source. Among these, *Stenotrophomonas maltophilia* was shown to colonize aquatic and terrestrial habitats. Our team recently observed the widespread distribution of *S. maltophilia* among soils sampled from various geographical areas and soil types (France, Tunisia and Burkina-Faso) including a gradient of heavy metal contaminants. In this work, we aimed at defining the state of these environmental strains regarding their virulence and antibiotic-resistance properties, in order to clarify issues about the origin of these properties among clinical strains. A panel of 55 distinct *S. maltophilia* genotypes isolated from these soils was selected and compared against 23 clinical ones obtained from various pathologies including pulmonary tract infections of cystic fibrosis patients and sepsicaemia. The following virulence properties were analysed: motilities (swimming, twitching, swarming), adhesion and biofilm formation, and enzymes secretion (e.g. caseinase, esterase, phospholipase C) at 28° and 37°C. Analyses were also performed on the *Dictyostelium discoideum* infection model which is based on its bacterial grazing ability. The antibiotic resistance profiles were obtained by the VITEK 2 system. Statistical co-inertia between a principal correspondence analysis on quantitative virulence traits, and a multiple correspondence analysis on qualitative antibiotic resistance traits and *D. discoideum* grazing deficiency was performed. The results showed a large diversity of virulence and antibiotic resistance traits in all the environmental and clinical strains tested. The main statistical effects linked motilities, antibiotic resistance profiles and *D. discoideum* grazing deficiency was performed. The results showed a large diversitiy of virulence and antibiotic resistance traits in all the environmental and clinical strains tested. The main statistical effects linked motilities, antibiotic resistance profiles and *D. discoideum* grazing deficiency. The clinical strains were differentiated by their multiple antibiotic resistances, a limited virulence against *D. discoideum* and higher flagellar motility. Most of the environmental strains were qualified as more susceptible to antibiotics and more virulent on *D. discoideum* than the clinical ones. Hence, a significant link related the high susceptibility of environmental strains towards antibiotics with the pathogenic effects on *D. discoideum*. Our work suggests that outdoor and clinical opportunistic pathogens have selected different evolutionary trade-offs to maintain either antibiotic resistance in a clinical context or virulence properties against amoeba in the environment.

352A DNA markers for detection and infrasubspecific discrimination of prevalent bovine mastitis-causing *Streptococcus*
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Bovine mastitis refers to an inflammatory disease in the mammary gland, affecting dairy herds worldwide. Due to changes in milk quality and composition, it is responsible for significant financial losses in the dairy industry. Over 150 etiological agents have been identified, with particular prevalence of *Streptococcus* spp. Thus, fast and efficient detection and typing methods are required for disease prevention, source tracking, treatment and control. In fact, recent studies have suggested the emergence of streptococcal lineages with decreased susceptibility to routine antibiotic treatment, which might impose an increased threat to dairy herds.
The aim of this study was to develop a fast, reliable and efficient platform for the detection of prevalent pathogens within the Streptococcaceae family, and gain additional insight into the infrasubspecific diversity of this group.

Using Insignia (http://insignia.cbcb.umd.edu), DNA signatures were selected for well-known bovine mastitis-causing taxonomic ranks: a broad spectrum marker for the Streptococcaceae family (F1), a taxa-specific marker for the Lactococcus genus (LC2) and specific markers for Streptococcus agalactiae (A1 and A2) and Streptococcus uberis (SU). Additionally, markers of functional traits associated with the pathogenicity of bovine mastitis strains were used: two markers from the fructose operon (FO1 and FO3), two markers from the nisin operon (NU1 and NU3) and two additional markers of virulence-associated genes, frequently described in Streptococcus uberis strains (V2 and V3). Experimental validation was carried out by PCR and dot blot hybridization. A set of 44 reference strains and isolates, representative of the Streptococcaceae family and closely related organisms with matching hosts, was tested with the selected DNA markers.

The results obtained showed that the broad spectrum taxonomic marker (F1) was specific to the Streptococcus genus, and the markers selected for Lactococcus (LC2), Streptococcus agalactiae (A1 and A2) and Streptococcus uberis (SU) were shown to be specific to the corresponding taxa. The functional markers provided further insight into strain-specific patterns of Streptococcus agalactiae and Streptococcus uberis: the fructose operon markers (FO1 and FO3) were specific to bovine isolates of Streptococcus agalactiae and the nisin operon markers (NU1 and NU3) were detected in a particular cluster of strains with a common geographic origin. Furthermore, dot-blots using the virulence-associated markers (V2 and V3) revealed specific patterns that were able to discriminate additional species, such as Streptococcus bovis and Streptococcus parauberis, and detect other organisms closely related to the Streptococcaceae family.

These data indicate that the combined use of taxa-specific and functional markers presents a promising approach for the reliable, rapid and efficient detection and typing of bovine mastitis-causing pathogens, with implications for community profiling of streptococcal species responsible for this disease.

### 353A Gene regulation and the cost of antibiotic resistance

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Resistance genes generally carry a fitness cost due to pleiotropic effects or costs associated with the expression or action of resistance genes. In order to predict the persistence and spread of antibiotic resistance it is important to understand the costs and the benefits of resistance. Many resistance genes are not expressed constitutively, but are induced by the antibiotic. One example is the tetRA operon that confers resistance to the translation inhibitor tetracycline. The regulator TetR represses the expression of both itself and the tetracycline efflux pump TetA. tetA is only expressed when tetracycline enters the cell and binds to TetR, thereby releasing the repressor. In this case the costs of the resistance gene and the benefits conferred by it are directly linked through this regulation.

To disentangle and quantitatively measure the costs and benefits of tetA expression a transcriptional tetA-gfp fusion was cloned behind the arabinose inducible promoter P_{BAD} on plasmid pBAD24. This allows for tight regulation of tetA expression and the simultaneous measurement of expression levels. This expression vector was transformed into an ara- strain of Escherichia coli. Replicate cultures were grown in 96-well plates and optical density and GFP-fluorescence was recorded in regular intervals.

We present quantitative fitness costs and benefits of the tetracycline efflux pump TetA and their dependence on the level of expression of tetA as measured by relative growth rate.

Information on the nature of the costs and benefits of antibiotic resistance at varying levels of antibiotics and its link to the expression of resistance genes are important to our understanding of the evolution and spread of antibiotic resistance.
Coral Black Band Disease (BBD) was the first coral disease to be documented; more than three decades later, BBD is considered a well-described, but as yet, unresolved disease plaguing corals worldwide. To date, a primary pathogen has not been characterized and the disease is believed to be caused by a complex polymicrobial consortium. In this study we demonstrate that the microbial communities associated with necrotic tissue of BBD-affected coral colonies are diverse and dynamic, and unique to specific seasonal time-points and coral colonies. In the highly-active (summer) stage BBD samples showed low microbial richness and diversity; a higher microbial richness and diversity was evident in the dormant (winter) phenotype in the same corals the following winter. Two main components of the BBD mat investigated here include cyanobacteria and vibrios. Cyanobacteria-OTU (99% homologous to Pseudoscillatoria coralii) in the BBD mat was several orders of magnitude higher than those in the healthy-apparent tissues of the affected corals. P. coralii cells showed temperature-independence and dominated the BBD-affected coral samples in both highly-active and dormant black band phenotypes – indicating that infected coral skeleton may serve as a reservoir for BBD cyanobacteria. In addition, Vibrio-clones associated with highly-active bands were similar to known pathogens, while dormant clones were similar to general aquatic Vibrio sp. Cultured BBD isolates of Vibrio sp. were highly homologous to previously documented BBD-associated vibrios from the Caribbean, Bahamas and Red Sea, as well as to other known coral pathogens, and showed typical proteolytic activity, which correlated with water temperature elevation.

Glycans on mucosal surfaces play an important role in host-microbe interactions. The B4galnt2 gene encodes a blood-group-related glycosyltransferase that is subject to strong selective forces in natural house mouse populations, whereby a common allelic variant exists that results in loss of B4galnt2 gene expression in the gastrointestinal tract. We hypothesize that altered glycan-dependent intestinal host-microbe interactions may underlie these signatures of selection. We previously identified significant changes in composition of the intestinal microbiota with respect to B4galnt2 genotype, indicating a previously unappreciated role for B4galnt2 in host-microbial homeostasis. In addition, we detected numerous B4galnt2-dependent differences in the abundance of specific bacterial taxa, including species belonging to the genus Helicobacter, suggesting interaction with B4galnt2 glycans. To determine whether B4galnt2 expression also influences host susceptibility to enteric pathogens, we have applied a model of systemic infection with Salmonella enterica serovar Typhimurium. Based on intestinal colonization and cecal histopathology, we find a significant influence of B4galnt2 genotype during the early phase of infection. Furthermore, infection was associated with dynamic changes in the intestinal tissue-specific expression pattern of B4galnt2. These results support the hypothesis that variation in B4galnt2 GI expression may alter susceptibility to diseases such as infectious gastroenteritis.

Giardia intestinalis is the most frequently identified protistan cause of intestinal infection in the U.S. and worldwide. The essential role of commensal microbes in immune homeostasis and pathogen susceptibility inspired us to survey Giardia-induced perturbations to the small intestinal bacterial communities. Comparisons of bacterial abundance in both Giardia-infected and uninoculated murine small intestines demonstrate distinct shifts in the bacterial community makeup. During giardiasis, the overall abundance of bacteria in the proximal small intestine increases an average of two-fold, with a
significant expansion of the Enterobacteriaceae. This work is the first evidence of a major shift in the diversity and abundance of the small intestinal microbiome as the result of Giardia infection. The Enterobacteriaceae, which are known to bloom in response to inflammation and oxidative bursts, are sensitive indicators of redox potential and inflammation within the gastrointestinal environment. This population of bacteria is known to outgrow during inflammatory bowel disease, a frequent sequela to giardiasis. Subtle inflammatory changes can provide novel electron acceptors within the chemical environment of the intestine, which can alter the composition of the commensal microbiota. Analysis of lipid peroxidation within the small intestines of infected mice shows significantly increased malondialdehyde levels after 7 and 14 days of infection, suggesting that Giardia colonization increases oxidative stress and changes the redox state of the small intestine. In addition, antibiotic pretreatment is known to enhance Giardia infectivity. To further query the role of the microbiota in mediating giardiasis, mice were infected with Giardia after pretreatment with an antibiotic cocktail or a saline control, and sacrificed at days 3, 7, and 14 post-infection. The small intestinal mucosal surfaces and luminal contents, as well as cecum contents and fecal pellets, were sequenced with Illumina MiSeq high-throughput 16S amplicon sequencing. Studies of the microbial ecology in the infected intestine not only inform our knowledge of the host-microbiome relationship, but also provide a sensitive tool for elucidating the dynamics of host-pathogen interactions. Future work will elaborate on micro-inflammatory cross-talk between Giardia trophozoites, the intestinal epithelium, and the commensal microbiota.

356A  The resident skin microbiota of the frog *Phylomedusa distincta* and the animal defense system against pathogens

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Anuran skin is an organ responsible for gas exchange and defense against pathogenic agents. The granular glands present on the frog skin produce bioactive molecules that are responsible for such protection. In addition, the skin harbors a resident microbial community but its role on the protection against pathogens is not known. Our working hypothesis is that the microbiota may act to complement the defense system against pathogen infection. The objective of this work was to describe the microbial diversity of the anurans dermis of the species *Phylomedusa distincta* using molecular methods. Twelve animals were collected at the State Park of Intervales located in São Paulo, Brazil. The microbiota on the skin of each frog was collected with a sterile swab, after the animal was washed three times in distilled sterile water. DNA was extracted for an initial analysis by DGGE. Three samples were randomly selected for the construction of 16S rRNA gene libraries. For each library an average of 60 sequences of approximately 400 bp were analyzed. The DGGE revealed the presence of few bands, ranging from 1 to 15 bands per sample, suggesting a low richness and indicating that a clone library analysis would be appropriate to cover the diversity. None of the bands was present in all samples; the band with the highest prevalence was observed in 83% of the samples, which indicated a high individual variability of the microbiota composition on the frog skin. The sequence analysis of the 16S rRNA gene libraries revealed a predominance of the γ-Proteobacteria Class belonging to the family Enterobacteriaceae, other families such as Pseudomonadaceae and Xanthomonadaceae were present in lower abundances. These results were in accordance with DGGE indicating that the microbiota on the skin of *Phylomedusa distincta* presented low richness. In addition, coverage values and rarefaction analysis indicated that the bacterial diversity was sufficiently covered with the number of sequences analyzed. On the other hand, the high individual variability initially observed was found to be false, at least at the family level, since Enterobacteriaceae was observed to be predominant in all animals analyzed by 16S rRNA clone libraries. Although this bacterial family is commonly found in association with animals, they are abundant not on the skin, but in the intestines of animals. On the other hand, some members of the family Entero bacteriaceae produce antimicrobial molecules that are effective against other Enterobacteriaceae, for example: colicins, microcins, and bacteriocins, which is consistent with the hypothesis that the microbiota of the frog dermis may be a part of the defense system against certain pathogens.
Survival and leaching of Tetracycline resistant bacteria and E. coli from manure in field scale experiments

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The spreading of manure on agricultural land is an economic and practical solution for improving soil nutritional and structural quality; however, animal manure frequently contains zoonotic pathogenic bacteria, such as certain Eschericia coli, Salmonella spp. and Campylobacter spp. The present experiment was conducted as a large multidisciplinary project. Pig manure with a natural content of Tetracycline resistant bacteria and E. coli was followed in a field scale experiment.

Pig manure was injected into agricultural soil and the distribution and survival of bacteria around the manure slurry slit in the soil was followed. During a period of two months, sections of soils with different distance to the manure string were assayed to obtain information on survival and spread of E. coli and Tetracycline resistant bacteria. The die-off of the different organisms was quantified showing an extended survival close to the manure string. Genomic DNA from 400 Tetracycline resistant bacteria was isolated and their phylogenetic relationship was established using BOX PCR showing that the main Tetracycline resistant bacterial species is E. coli.

Drainage water from the field sites were collected weekly from one year prior to manure application, where no Tetracycline resistant bacteria were detected. For a period of 11 months following the first manure application, drainage water was sampled proportional to the flow and collected weekly. Selected storm events were intensively monitored by the collection of subsamples for every 2 mm of drainage runoff, using a refrigerated ISCO sampler. Drainage samples were tested for Tetracycline resistant bacteria, E. coli, conductivity, turbidity, Cl and Br. The highest concentration was found in the first drainage sample following manure application and a fast decrease in cell numbers in the following drainage samples was seen. For the Tetracycline resistant bacteria concentrations exceeding 100 CFU ml⁻¹ was detected.

In conclusion, the survival and environmental spread of antibiotic resistant organisms show that the upper soil and drainage water are impacted by a high load of antibiotic resistant bacteria originating from pig manure.

Attachment of E. coli and Salmonella species in runoff under influence of polyacrylamide

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Pathogens are released into the environment through animal waste, biosolids from wastewater and contaminated irrigation water. The spreading of pathogens is mostly determined by the presence of water and its flow paths. Rainfall and/or irrigation will partition between infiltration into the soil and runoff over the soil. US Environmental protection Agency (EPA) assessed 33% of U.S. waters in 2000 and found that 40% of streams, 45% of lakes, and 50% of estuaries were not clean enough to support fishing and swimming because of bacterial concentrations.

Polyacrylamide (PAM) is used in some agricultural systems to increase soil structure and hereby be able to minimize soil erosion with as much as 95%. It is applied either as a liquid through the irrigation water (typical concentrations range from 1 to 10 mg/L), or as a solid tablet or granule introduced in the bottom of the furrow.

In the present experiment four pathogens (E. coli O157:H7, TSV354 E. coli, S. Poona and S. Newport) were chosen based on differences in mobility and hydrophobicity. A single strain attachment study showed that PAM decreased the bacterial attachment in general, but attachment was also influenced by both soil type and bacterial strain.

The runoff experiment was made in trays (40 cm long, 7 cm deep, 10 cm wide) with a 5% slope, surface 2 hours prior to the onset of the run-off to allow adhesion between bacterial cells and soil particles. Watersamples were analysed for total suspended solids (TSS), dissolved suspended solids...
(DSS) and attached/unattached pathogenic cells by the buoyant-density separation procedure. We found that soil particle run-off is affected by the PAM treatments; however the soil type influences the degree of the effect. As for bacterial run-off there is a general increase in both planktonic and attached cells when the run-off water contain PAM.

For each treatment in the run-off experiment 32 colonies were randomly picked. DNA from isolated colonies was analyzed by Repetitive Extragenic Palindromic elements (REP) to determine differences between bacterial trains in each run-off treatment. S. poona was recovered less than the other strains.

359A Distribution and composition of Borrelia burgdorferi communities in tick populations across central Britain
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Lyme borreliosis is an emerging infectious disease of humans and animals in the United Kingdom, with cases reported from most counties in England and Wales. The causative organisms, which are members of the Borrelia burgdorferi sensu lato group of spirochaetes, have complex ecological relationships with their vector, Ixodes ricinus, and with their vertebrate reservoir host. Both the prevalence and composition of Borrelia communities have implications for public health risk. We have been monitoring B. burgdorferi communities in a number of sites with abundant tick populations across northern England, North Wales and the Scottish Border region. This region includes both areas with frequent reports of human infection, and areas where few cases are reported. We have quantified a remarkably patchy distribution of the pathogen in different questing nymphal populations and have identified potential determinants of this patchiness. Furthermore, we have explored the diversity of B. burgdorferi communities at different sites, encountering three species, B. afzelii, B. garinii and B. valasiana. However, the contribution of each species to local B. burgdorferi communities varied markedly between sites.

360A Reliability of qPCR for quantification of bacterial pathogens in highly PCR-inhibiting environments: Application of an integrative concept for Vibrio cholerae
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Quantification of bacterial pathogens via quantitative real-time PCR (qPCR) is a challenge in complex environmental samples and fundamental for ecological studies and risk assessment. Vibrio cholerae is an important human pathogen thriving in a variety of aquatic ecosystems. V. cholerae serogroups O1 and O139 are the causative agents of the severe diarrheal disease cholera, other serogroups cause otitis, gastroenteritis, wound and blood infections. Beside cultivation via plate counts or most probable number techniques, qPCR has the potential to provide reliable quantitative data and offers the opportunity to quantify multiple targets simultaneously. The method was chosen for the quantification of V. cholerae in highly turbid environments at Austria’s lake Neusiedlersee, where V. cholerae was shown to be an autochthonous component of the bacterial communities.

A novel triplex qPCR based on TaqMan probes was developed. Probes for quantification of total V.cholerae (ompW) and choleragenic V.cholerae containing the cholera toxin gene (ctxA) in water were designed. To identify possible inhibition by environmental factors, an exogenous internal amplification / extraction control was included in this assay. The specificity of this assay was tested against a panel of 86 bacterial isolates. Only V. cholerae strains verified to contain ompW and/or ctxA gene generated a fluorescent signal, confirming the specificity of the assay. The assay was quantitative across the tested 5-log dynamic range down to a method detection limit of 5 genomic units/rxn with a minimal efficiency of 96%. Repeatability was lower than Cq0.29 and reproducibility was lower than 11% for all three tested target genes. Possible inhibiting effects based on unequal target gene concentrations were controlled with matrix assay, where no significant influence could be detected.

V. cholerae type strain O395 was used for spiking assays in natural lake water, recovery rates were dependent on the environmental background ranging from 16 up to 29%. For the first time, we could
successfully quantify *V. cholerae* via qPCR even in extremely turbid environmental water samples in combination with an internal control. We suggest our triplex qPCR as a powerful tool to quantify and differentiate choleragenic from non-choleragenic *V. cholerae* in ecological studies as well as for risk assessment and monitoring programs.

**361A Some epidemiological factors related to occurrence of shiga toxin producing E. coli (STEC) in calves, cattle and man in some Egyptian localities**

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Livestock animals especially cattle seem to be the major reservoir for STEC characterizing them as a zoonotic pathogens. A total of 863 fecal and stool samples were collected from 264 calves, 281 cattle and 318 human in some rural areas of Behera and Menoufia Governorates, Egypt. All samples were cultivated on MacConkey's sorbitol agar media. Suspected colonies were examined by vero cell assay (VCA) to reveal their cytotoxic effect. Positive samples were serotyped and further confirmed by PCR to determine the type of Stx.

Out of 264 fecal samples from calves, STEC was detected in 15 (5.68%) samples as examined by VCA. The use of multiplex PCR, revealed that 14 (5.30%) out of 15 VCA positive strains were confirmed as STEC. Among the 14 confirmed strains, 3 strains (1.14%) were serotyped as O157. Occurrence of STEC appeared to be higher in young, diarrheic bull calves than in old, apparently healthy cow calves. Occurrence of STEC seems to be related also with the level of farm hygiene and type of feeding calves. Among 281 fecal samples from cattle, STEC was detected in 12 (4.27%) samples using VCA and all positive samples were confirmed by multiplex PCR. O157 serotype was detected only in 2 samples 2 (0.71%). Occurrence of STEC was higher in young, diarrheic cattle kept under bad standard of hygiene than in old, apparently healthy cattle kept under good level of hygiene. Moreover, detection of STEC appeared to be higher in animals during warmer than cooler season.

Occurrence of STEC in stool of some people in the investigated localities revealed that among 318 stool samples, STEC was detected in 7 (2.2%) samples as examined by VCA. Six strains (1.89%) out of seven were confirmed using multiplex PCR and two strains (0.63%) were serotyped as O157. In the same manner, occurrence of STEC appeared to be related with the young, diarrheic and males than old apparently healthy and female individuals. People with history of animal contact had higher frequencies of STEC detection than non contacts. The overall sensitivity of SMAC for detection of STEC in calves, cattle and human samples compared with VCA was 36.96%, while the overall sensitivity of PCR for detection of STEC in comparison with VCA was 94.29%. In addition to the rapidity, PCR possessed higher sensitivity than that of SMAC and VCA in detection of STEC.

**361B Mucin-2's role in the potential inhibition of probiotics in meningitic Escherichia coli K1 E44 adherence to intestinal epithelial cells**

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The human intestinal tract is a complicated ecological system in which more than 800 species of microbiota inhabited. A physicochemical barrier of the intestinal tract consists of the intestinal epithelial cell layer and the mucin protective layer produced by goblet cells. Intestinal tract mucins perform an innate protective function against microbial invasion. But currently, the interaction between probiotics/pathogens and mucins is little known. In order to determine the effects of probiotics on the upregulation of mucin-2 and the subsequent inhibition of Escherichia coli K1 attachment and invasion to intestinal epithelial cells, the mucin-2-knockout Caco-2 cells model was established with RNA interference and used to examine probiotics/pathogens-mucin-2 interaction.

First, mucin-2 knockdown experiments were carried out in Caco-2 cell line. The mucin-2 siRNA oligonucleotides sequences which include 4 mucin-2 small hairpin fragments were cloned into pGPU6/GFP/Neo expression vector, while the vector alone and the one with an insert containing green fluorescence protein (GFP) were used as the controls. The knockdown of mucin-2 allows us to
evaluate its impact on probiotics/pathogens adhesion to intestinal epithelial cells. Then, these shRNA was carried on a pGPU6/GFP/Neo-shRNA expression vector to facilitate their transfection and expression. Real-time PCR was used to detect mucin-2 mRNA in transfected cells at 24h and 48h after transfection, respectively. Finally, the effect of mucin-2 knockdown on Escherichia coli E44 adhere to Caco-2 in the presence of probiotics was examined. The viable bacteria count was applied to determine the number of colony form unit of Escherichia coli adhesion to Caco-2 cells that were incubated with probiotics. Results are presented as the percent adhesion of inoculum: [(number of Escherichia coli recovered)/(number of Escherichia coli inoculated)] x 100%.

The results showed that anti-mucin-2 shRNA is an effectively way to silence endogenous mRNA and thus mucin-2 proteins in Caco-2. Those shRNAs located inside the open reading frame of mucin-2 were able to reduce mucin-2 mRNA level significantly, and as a control, vector bearing GFP or untreated Caco-2 revealed normal level of mucin-2. In the case of mucin-2-knockdown Caco-2, the adhesion ability of Escherichia coli E44 in the presence of probiotics almost reached to the same level as control (0.72±0.47 vs 1.00±0.00, P>0.05), but the relative adhesion of E44 in the probiotics+pathogens group was 0.40±0.18 vs 1.00±0.00 (P<0.01) in the normal Caco-2. In brief, regardless of the interference efficiency (60.2%), our results suggest that mucin-2 plays an important role in the regulation of probiotics against bacterial adhesion to Caco-2 cells, that is the effects of probiotics on the upregulation of mucin-2 and the subsequent inhibition of Escherichia coli K1 attachment to intestinal epithelial cells.

362A Ecological diversity of cereulide-producing strains of Bacillus cereus and Bacillus weihenstephanensis

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Bacillus cereus is an important foodborne pathogen causing diarrhea or emesis and even lethal poisoning. The emetic syndrome is caused by the production of a heat-stable dodecadepsipeptide toxin, the cereulide. Cereulide-producing strains of B. cereus are known to be a group of closely related strains. The aim of this work was to study the diversity of these strains in food and environmental niches and to gather more insights into the ecology and epidemiology of these strains.

A set of fifty-two cereulide-producing strains was used in this study. They were isolated from foods, emetic-type food poisoning cases and environmental niches (soil, mammals and rodents), as previously described by Hoton and coll. (2009). They originated from ten countries (Belgium, China, Denmark, France, Germany, Ivory Coast, Poland, Switzerland, United Kingdom and United States) and were isolated, in majority, between 2003 and 2011. The genotypic and phenotypic characteristics of these strains were studied using biochemical tests (API20E and API50CH) and molecular methods (Pulsed Field Gel Electrophoresis, PFGE).

The phenotypic study confirmed the existence of two distinct clusters among emetic strains. Indeed, the majority of strains were unable to degrade starch and glycogen (Cluster I) while members of the cluster II were positive. The diversity within the emetic strains was also assessed by comparing their lecithinase and haemolysis activities. All the strains were positive for these two tests except three strains. These phenotypic traits are important since the key diagnostic features of B. cereus are based on these activities. In routine detection, these pathogens strains cannot be considered as B. cereus and this can lead to misidentification and underestimation of foodborne illnesses caused by these bacteria.

The characterization by PFGE could discriminate 17 different profiles from a total of 52 strains analyzed. Several striking observations could be made from these results. First, emetic strains isolated from the same outbreak in different matrices gave rise to different pulsotypes. Secondly, the number of strains involved in food intoxications is limited and this group of clonal (or undistinguishable by PFGE) strains frequently occurred in successive outbreaks. Finally, the pulsotyping of random isolates from different countries (geographically remote) displayed the same profile. For instance, Belgian food emetic strains were found to share the same profile with two Chinese strains isolated from ice creams.
Taken together, these data demonstrate the importance to perform a systematic characterization of all emetic strains by PFGE. This is essential for further epidemiological studies and to gather important information on their potential reservoirs.

363A High abundance of pathogenic bacteria in Kuala Sepetang tropical mangrove estuary, Malaysia
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Kuala Sepetang mangrove estuary, located on the west coast of peninsular Malaysia facing the Malacca Strait, supports a wide variety of marine organisms due to its warm tropical weather and healthy mangroves providing protective nursery grounds for fishes, crustaceans and mollusks. It serves as important breeding grounds for various commercial aquaculture stocks in Malaysia. However, several national reports had indicated frequent pathogenic vibrios contamination in the exported seafood. Therefore, this study was undertaken to study the ecology of various potential human pathogens, particularly Vibrio cholerae in Kuala Sepetang estuary and their association with copepods and aquaculture activity. In this study, estuarine water, sediments and copepods were sampled from eight stations located along three main rivers of the estuary: Kuala Sepetang River (1 station), Selinsing River (2 stations) and Sangga Besar River (5 stations). Along Sangga Besar River, two fishing villages, a commercial cockle breeding spat and fish cages could be found. Kuala Sepetang and Selinsing River were away from human activity, served as control in this study. The samples were enumerated and isolated for fecal coliforms, *Escherichia coli*, *Enterococcus* sp., *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Staphylococcus* sp. and *Bacillus* sp. using conventional culturing method. Fecal coliforms, *Escherichia coli* and *Enterococcus* sp. were detected in all stations, showing no significant differences in their densities between contaminated and control stations. *Vibrio parahaemolyticus* was highly abundant in Kuala Sepetang estuary (2.6±0.6 log CFU/100ml of estuarine water; 3.2±0.8 log CFU/g of sediment). No apparent correlation was observed between *Vibrio parahaemolyticus* density and the physical parameters in the water column. Unlike *Vibrio parahaemolyticus*, correlation with environmental parameters revealed that salinity, redox potential and pH were important factors governing *Vibrio cholerae* densities in the estuary. Although statistically non-significant, *Vibrio cholerae* densities were obviously higher in the human-impacted river (Sangga Besar River; 2.9±0.5 log CFU/g) than those away from human activity (2.1±0.8 log CFU/g). Noteworthy, station E located in front of a fishing village and with numerous fish cages around, had lower counts of fecal indicators, *Vibrio parahaemolyticus* and *Vibrio cholerae* compared to other stations along Sangga Besar River throughout the study period. However, highly pathogenic *Vibrio cholerae* O1/0139 were isolated from station E. More studies are required to investigate if the fish cage practice had shaped this unique bacterial population at station E. On the contrary to *Vibrio cholerae*, *Bacillus* sp. and *Staphylococcus* sp. were detected more frequently in the estuarine water and sediments from Selinsing River with less human activity. *Aeromonas* sp. and *Vibrio* sp. were predominant colonizing pathogens in copepods isolated from Kuala Sepetang estuary. *Staphylococcus* sp., *Pseudomonas* sp. and *Stenotrophomonas* sp. were isolated at lower frequencies from copepods. It was concluded that Kuala Sepetang mangrove estuary supports a great number of potential human pathogens. The occurrence of *Vibrio cholerae* was governed by environmental parameters and human activity. More studies are required to better understand the ecology of these pathogens in the tropical mangrove estuary.

364A The effect of whole-grain compared to refined wheat on the gut microbial composition and integrity in a colonic epithelial cell model following a 12-week energy-restricted dietary intervention in postmenopausal women
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Intake of whole-grain products are considered to decrease the risk of cardiovascular disease (CVD). This effect could potentially be linked to a prebiotic effect, hence positive modulation of the gut microbial composition or activity. Kristensen and coworkers recently conducted a study in postmenopausal women who were randomized to either whole-grain wheat (WW) (n=38) or refined wheat (RW) (n=34) consumption as part of an energy-restricted diet for 12-weeks following a 2-week run-in period with RW. Percentage fat mass as well as serum total and LDL cholesterol were found to
differ between the two groups (Kristensen, et al, 2012). We used fecal samples from the same study to examine effects of WW and RW on the bacterial composition by quantitative PCR targeting the phylums **Bacteroidetes** and **Firmicutes**, and the genera **Bifidobacteria**, **Lactobacillus**, **Bacteroides**, and **Prevotella**, as well as the **Enterobacteriaceae** family. Potential bifidogenic effects were examined in depth by determining the levels of **B. bifidum**, **B. adolescentis**, **B. catenulatum**, and **B. longum**. The ratios of both **Bifidobacteria** and **Lactobacillus** increased following the WW intervention.

The composition of the gut microbiota may affect the intestinal integrity, which in this study was evaluated *in vitro* by determining transepithelial resistance (TER) across a Caco-2 cell monolayer. Fecal water collected after the run-in period and following the intervention period for 26 participants (WW; 15 participants, RW; 11 participants) were used to determine effects of WW, RW, and microbiota composition on TER. Preliminary results indicate that fecal water from WW and RW both before and after intervention in general had a positive effect on TER, however, there was no difference in TER between WW and RW. Correlations between microbial composition and effect on intestinal integrity indicated a negative correlation between **Bifidobacteria** and TER as well as a positive correlation between the **Firmicutes**/**Bacteroidetes** ratio and TER.

365A  **Intestinal colonization, gut function and inflammatory responses are moderately influenced by gestational age at birth**

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Suboptimal intestinal colonization is observed for many infant diseases, but it remains unknown if the divergent microbiota is mainly a result of disease or inborn characteristics such as maturational stage at birth, immune development and gut function. Preterm birth alters the gut microbiota which is considered a risk factor in necrotizing enterocolitis (NEC), a severe intestinal disease of preterm infants. However, it is unclear if gestational age or rather hygiene practices and antibiotic use determine the colonization differences between term and preterm newborns.

We hypothesized that gestational age at birth determines early gut colonization and immune development and that pigs born at different gestational ages but raised in the same environment differ in gut colonization, immune development and gut function.

Pigs were delivered by cesarean section at day 100 (n=4), 105 (n=18), 110 (n=14), and 115 (considered term, n=21) of gestation. Except for d100 pigs, all fed cow's colostrum, the groups were divided into diet groups and fed either formula or cow's colostrum via oro-gastric catheters. On day 3, all pigs were euthanized for tissue collection. Small intestinal microbiology was assessed by 454-sequencing of bacterial 16S rDNA purified from luminal contents and subsequent analysis in the CLC Bio software. Totally 38 different bacteria were detected and of these only 11 were dominating (relative abundance >0.01). Gestational age minimally affected gut colonization but colostrum-feeding tended to increase Clostridium perfringens and Streptococcus sp. but decrease Lactobacillus sp across gestational ages. Bacterial abundance in nine sections along the small intestine was assessed by fluorescence in situ hybridization using a 16S rRNA probe targeting the Bacterial Domain. Across sections and irrespective of diet, bacterial abundance was significantly reduced in d115-pigs compared to the preterm groups (P<0.05). For all age groups, bacterial abundance nearly doubled in the mid and distal sections compared to the proximal. Small intestinal expression of genes related to immune function and gut maturation was assessed by high throughput qPCR using a 48.48 Dynamic Array (Fluidigm) for 2304 simultaneous reactions. We used replicates of 26 primer pairs inclusive reference genes and found up-regulation (fold change >2) of genes related to pathogen sensing and inflammation (Il-1β, Il-8, NfκB and TLR2), barrier and gut function (MUC2, iAP, CLDN3, occluding and SGLT1) in d115-pigs and in some cases d110-pigs compared to pigs born more preterm. Across all ages, up-regulation of Il-8, TLR-4, TLR-2, C3 and MUC2 and down-regulation of iAP and lactase was observed after formula-feeding compared to colostrum-feeding. The dietary influence on gene regulation was most pronounced in the d105- and d110-pigs while it was almost absent in d115 pigs.

Although gestational age only affected gut colonization in regards to bacterial load this may affect sensitivity towards diseases such as NEC. The altered gut immune function possibly reinforces this sensitivity also through effects on the following colonization processes. However since diet affected
gut colonization, immune development and gut function, dietary interventions may be used as a tool to counteract possible adverse effects of preterm birth.

### 366A The soil resistome: The anthropogenic, the native, and the unknown

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Soil contains extremely high levels of antibiotic resistant bacteria (ARB), which harbor a diverse array of antibiotic resistant genes (ARGs). It has been previously hypothesized that many ARGs associated with human pathogens originated in antibiotic-producing soil bacteria, and that novel soil-associated ARGs can potentially reach clinically relevant bacteria via horizontal gene transfer, potentially resulting in severe future epidemiological ramifications. Over the past decade it is becoming increasingly clear that anthropogenic activities can expand environmental ARG reservoirs, and therefore practices such as animal husbandry (in cases where antibiotic are administered) and manure amendment have been the focus of a wide array of studies. This presentation presents data from two comprehensive studies in attempt to provide a holistic overview of how external and intrinsic factors influence the soil resistome. The first study addresses the impact of treated-wastewater (TWW) irrigation, which potentially results in proliferation of AR in irrigated soil microcosms due to release of residual antibiotic compounds, ARB, and ARGs; while the second assessed the scope of known and novel ARGs in native, non-agricultural “pristine” soils. Both experiments employed standard culture-based isolation methods and culture-independent molecular analyses such as metagenomics and quantitative real-time PCR (qPCR). Although high levels of ARB and ARG were detected in both freshwater (FW)- and TWW-irrigated soils, and despite the fact that the TWW itself contained relatively high levels of ARB, no significant differences were seen when comparing the resistance profiles of the FW- and TWW-irrigated soils. Additionally, irrigation of bench-scale soil mesocosms with water or media containing residual and even clinically-relevant antibiotic concentrations did not significantly alter ARB levels or bacterial community composition further suggesting that anthropogenic effects seem to have minor impact on the soil resistome. In contrast, analyses of several physiochemically distinct “pristine” soils revealed a wide array of phylogenetically-diverse ARB, harboring both novel and clinically-associated ARGs. The later included genes associated with next-generation antibiotic resistances, who to the best of our knowledge have not been previously detected in soil isolates. Combined, these data suggest that the soil resistome is extremely resilient to anthropogenic impact, but that native soil bacteria represent a highly diverse reservoir of ARGs which can potentially be transmitted to clinically-relevant bacteria. It also provides additional support for the theory that many clinically-relevant ARGs originate in soil bacteria.

### 366B Fine-tuned bistability stabilizes the cooperation-based intestinal virulence of *Salmonella*

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*Salmonella* Typhimurium (S. Tm) express costly virulence factors (VF) to elicit inflammation, a public good which aids the pathogen to outgrow the intestinal microbiota. By definition, such public good based cooperation is prone to be sapped by the invasion of defectors, i.e. mutants which never endure the cost of cooperation but profit from the public good. How S. Tm deals with defectors during infection was, until now, unclear. Among VF, the type three secretion system 1 (T1) is essential to trigger inflammation. Interestingly, from the same genome, S. Tm stochastically forms T1expressing (T1⁺) and T1 non expressing (T1⁻) cells. Switching from one state to the other occurs by a process called bistable gene expression. Thus only a part of the S. Tm population undergoes the cost of virulence expression. We therefore hypothesized that bistable T1 expression could stabilize cooperation by generating a subpopulation (T1⁺) excluding defectors. This was studied by computational modeling, within-host evolution experiments, genome re-sequencing and mutant analysis. Results observed in vivo demonstrated that *hilE*, a negative regulator limiting T1 expression to a small subpopulation of the S. Tm cells, was of key importance. Disrupting *hilE*, i.e., increasing T1⁻ cells proportion, accelerated
defector invasion to such an extent that inflammation subsided and the S. Tm population declined. This study shows how a fine-tuned bistable switch in gene expression allows a division of labor stabilizing cooperation-based virulence: T1+ cells produce the costly public good, while T1- cells compete against defectors.

367A The fpvB gene, encoding an alternative type I ferricyanide receptor, can be lost during long-term colonization of the cystic fibrosis lung by Pseudomonas aeruginosa
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The cystic fibrosis lung provides a well-suited environment for a broad range of microorganisms, among which Pseudomonas aeruginosa becomes the dominant pathogen when the host reaches an age of about 25 years. In order to extract iron out of the cystic fibrosis lung environment, Pseudomonas aeruginosa produces the high-affinity iron(III)-chelating molecule pyoverdine. Here, we report the use of multiplex PCR to map the ferricyanide receptor distribution among Pseudomonas aeruginosa isolates obtained from patients attending the UZ Brussels, Belgium.

Via multiplex PCR, the ferricyanide receptor type of 30 Pseudomonas aeruginosa isolates obtained from patients attending the UZ Brussels (Belgium) was determined. Further, sequencing of the complete fpvB gene and an internal of the mucA (from position 42 to 544) gene was performed. Finally, the pyoverdine production was determined by means of fotospectrometry.

In an attempt to type the ferricyanide receptors used by cystic fibrosis Pseudomonas aeruginosa strains, it was observed that the fpvB gene was present at a remarkably lower percentage (80%) in the total population investigated than what has been observed in literature (100% for cystic fibrosis isolates and 93 % for environmental isolates). Further, pyoverdine production was only observed in 70% of the isolates studied. This indicates that pyoverdine production and the fpvB gene could be potentially lost during long-term infection of the cystic fibrosis lung. This hypothesis was supported by the fact that Pseudomonas aeruginosa has alternative systems to acquire iron, such as haem (Ochsneret al., 2000) or ferrous ion transport systems (Lamont et al. 2009). In order to have an idea about the colonization time, the number of mutations in an internal fragment of the mucA gene, which is one of the most frequently mutated genes in Pseudomonas aeruginosa strains of cystic fibrosis patients, were used as a reference for the adaptation time of the isolates studied. Interestingly, isolates that harbored more mutations in mucA, probably corresponding to a longer colonization time, did not harbor the fpvB gene. In order to confirm the loss of the fpvB gene, primers were designed in a gene upstream and a gene downstream of fpvB and the subsequent PCR product was sequenced and analyzed. Next to the confirmation that a large portion (> 80%) of fpvB was deleted, the two genes surrounding fpvB showed no deletions or stop mutations. This finding indicates that a potential negative selection pressure, selects for an inactive fpvB gene, without affecting the neighboring genes.

Although it is known that Pseudomonas aeruginosa is able to use alternative iron uptake systems, apart from pyoverdine production, during colonization of the cystic fibrosis lung, a negative selection pressure, such as antibiotic treatment, selects for the loss of function of the fpvB gene.

368A Establishment of mass spectrometric profiling in Vibrio Population analyses
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Coastal areas of the North and Baltic Sea are threatened by potential pathogenic Vibrio species. So far, outbreaks were generally reported to occur in regions with elevated water temperature. But due to Global Warming, an infiltration of Northern European temperate waters by perilous vibrios can be assumed. Major human pathogenic species are V. cholerae, V. vulnificus and V. parahaemolyticus. These bacteria can be transmitted by contaminated water or seafood. Infections lead to fatal diarrheas, septicemias or gastroenteritides, and cases of death caused by V. vulnificus strains have recently been reported from Baltic Sea countries. This implies the significance and urgency of Vibrio population analyses.
In this study, we aim to establish a mass spectrometric method to distinguish *Vibrio* strains on the species level. In contrast to DNA-based methods, species identification by this matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) method can be performed cost and time effective. First of all, identification results have to be confirmed by ribosomal protein fingerprinting. Additionally, MALDI-TOF cluster analyses allow the detection of possible interspecific groups with pathogenic properties. Another aim is to get information on the geographical distribution of specific *Vibrio* populations. For this reason potentially pathogenic vibrios are mapped according to their sampling site and environmental conditions like salinity or temperature at the sampling time. The outcome will be a detailed report about the composition of *Vibrio* populations in the North and Baltic Sea. Hence, it will be possible to answer the question whether pathogenic vibrios are to be perceived as a serious threat for German coasts.

So far, over 600 potential pathogenic *Vibrio* isolates were collected from environmental samples of the North and Baltic Sea. Sampling took place all along the German Coast, from the Dutch border to the isle of Usedom near Poland. Reference mass spectra are acquired from all these strains. Hence, the MALDI-TOF database will be expanded by environmental vibrios, which in turn leads to more specific classification results of further *Vibrio* strains. In order to detect interspecific cluster, comparative analyses of MALDI-TOF and ribosomal protein fingerprinting are performed. Such a comparison has been already accomplished with *V. alginolyticus* strains isolated from the German Bight and it has been proven that MALDI-TOF can distinguish two interspecific *V. alginolyticus* groups which were identified by RNA polymerase beta unit sequence analyses. In context with this result, similar cluster should be expected in the case of potential pathogenic *Vibrio* species. Hence, mass spectrometric profiling by MALDI-TOF can be a huge improvement in *Vibrio* population analyses.

369A Interactions of the pathogenic bacterium Listeria monocytogenes with protozoa and plants: a way for the pathogen to succeed in the nature

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Listeria monocytogenes is a ubiquitous Gram-positive bacterium that causes a disease in humans and a wild spectrum of domestic and wild animals. While being a facultative intracellular parasite, L. monocytogenes is able to survive and to multiply in the environment. Isolation of L. monocytogenes from soil and vegetation in nonagricultural suburban territories was described suggesting that soil and vegetation are the natural environment where the bacterium persists when it is outside of the host. Interactions between L. monocytogenes and other members of soil ecosystems are not fully studied despite the importance of this issue for monitoring of the pathogen.

The aim of our work was to characterize interactions of L. monocytogenes with free-living protozoa and plants and to establish a potential of the pathogen to be delivered to mammals from soil ecosystems.

The fully virulent L. monocytogenes strain EGDe was used. The free-living ciliate Tetrahymena pyriformis and the lettuce (*Lactuca sativa*) and the cabbage (*Brassica oleracea*) were used as model protozoa and plants. T. pyriformis trophozoites were mixed with bacteria with MOI 1000:1 (bacteria/protozoa) in the LB broth and incubating at 28°C for up to 14 days. Calli grown in MS medium were infected by introducing bacteria into the medium. Alternatively, bacterial suspension was used to shed on calli. Bacterial-protozoan and bacterial-plant interactions were followed with bacterial routine plating and with transmission and scanning electron microscopy. Guinea pigs were used to study development of listerial infection.

Co-cultivation of L. monocytogenes with T. pyriformis caused extensive mortality and encystment in ciliates while it was favorable for bacteria, which increased their concentration in comparison with isolated bacterial culture. Guinea pigs that consumed water contaminated with L. monocytogenes infected protozoan cysts developed listerial infection similar to the infection caused by bacterial suspension.
L. monocytogenes was found to be able to enter plant cells from the contaminated medium. The cytotoxic effect was observed that caused plant loss within 14 days. Being shed on calli, L. monocytogenes established biofilms on the surface. L. monocytogenes demonstrated stable growth in association with plants independently on a way of infection.

Overall, obtained results demonstrated that L. monocytogenes effectively interacts with T. pyriformis and studied plants that might give her benefits for survival. Furthermore, non-mammalian members of natural ecosystems might be factors of bacterial delivery to mammals thus supporting the circulation of the pathogen in the nature.

370A Evaluation of the virulence of the fish pathogen *Vibrio anguillarum* using a gnotobiotic sea bass (*Dicentrarchus labrax*) model

In this study, 16 wild-type *V. anguillarum* strains, isolated from different hosts (sea bass, trout, salmon,...) or ecological niches (sediment, water,...) were studied. Isolates were clustered according to their genetic fingerprint, established with both rep-PCR and RAPD methods. Interestingly, the clustering didn’t exactly follow the classification into the different serotypes. Virulence of the isolates was assessed using a standardized gnotobiotic model system with axenic European sea bass (*Dicentrarchus labrax*) larvae as hosts. Larvae were challenged with 10^5 colony forming units of bacteria per ml of water, and larval survival was monitored by microscopical analysis at regular intervals.

Significant differences were observed among the 16 tested *V. anguillarum* isolates. Eleven strains caused a significantly higher mortality compared to the axenic control. No clear relation could be established between virulence in the sea bass larvae test and serotype, presence of a virulence plasmid, or genetic fingerprint. In contrast, a relation could be established with the original host from which the strains were isolated as the five avirulent strains were all originally isolated from rainbow trout, suggesting a strong host specificity of the *V. anguillarum* virulence mechanism.

371A Adaptation to lungs of cystic fibrosis patients leads to reduced virulence and lowered resistance against natural protist and phage enemies in *Pseudomonas aeruginosa*

Pathogenic life style can lead to highly specialized interactions with host species. In extreme case, permanent association with host can select for less virulent pathogens that lose some of their virulence genes due to immune evasion. It is still however unknown how this kind of adaptation affects survival of opportunistic bacterial pathogens in external environments. Here we studied how *Pseudomonas aeruginosa* adaptation to the lungs of cystic fibrosis (CF) patients affects its virulence and survival in the presence of several, natural phage (14/1, ΦKZ, PNM and PT7) and protist (*Tetrahymena pyriformis* and *Acanthamoebae polyphaga*) enemies. We found that specialization to CF-lung environment favoured slowly growing small *P. aeruginosa* colony variants that showed reduced acute virulence in wax moth host (*Galleria mellonella*). While most clinical, non-lung isolates were innately phage-resistant and highly toxic for protists, CF-lung specialists were poor at both killing...
protists and resisting phages. These results show that \textit{P. aeruginosa} adaptation to lungs of CF-patients can lead to lowered acute virulence and greater susceptibility to natural phage and protist enemies. Reduced bacterial survival and infectivity could in turn lead to poorer transmission when pathogens are released to external environment from their hosts. Furthermore, our study suggests that phage therapy could offer potential alternative for antibiotics in treating chronic infections that are often characterized by multidrug resistant bacteria: chronic strains seems to be very sensitive to phages, while reduced growth and small population sizes will slow down the emergence of beneficial resistance mutations.

371B \textbf{Anthropogenic impacts on selection for antibiotic resistance in the soil environment}

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It is well known that the environment represents a reservoir of mobile resistance genes and mobile genetic elements associated with their transfer. However, it is less well understood how human behaviour impacts dissemination of resistant organisms in environmental compartments or how pharmaceuticals and other anthropogenic bioactive compounds may influence selection and gene flow.

Antibiotic residues are present in sewage sludge and animal faecal slurries in measurable concentrations, and in some cases at levels that have discernible impacts on microbial processes. In terms of antibiotic resistance, there are two main factors, dissemination and selection; disentangling these is complex but is important to understand the role of the environment in the evolution of resistance more fully. In-situ selection in the environment is potentially significant in that recruitment of novel genes into human associated bacteria may be facilitated. In addition to direct selection by antibiotic residues, indirect co-selection may occur by other bio-active compounds or elements such as biocides, detergents and metals. Detergents and biocides are present in impacted environments at relatively high concentrations (mg/l) where they may select for specific resistance mechanisms such as quaternary ammonium compound (QAC) resistance genes carried on clinically significant genetic elements such as class 1 integrons which are able to integrate and excise a wide range of antibiotic resistance genes.

Data are presented on prevalence of class 1 integrons in a range of soil environments, including those impacted by sewage sludge, animal faecal slurries, antibiotics and metals. In all cases samples were taken from plots with adjacent controls. Pristine or semi-pristine soils were also analysed as additional controls. Real-time PCR was used to estimate molecular prevalence of class 1 integrons and QAC genes. Functional metagenomic libraries were constructed from a subset of samples and screened for extended spectrum $\beta$-lactamases (ESBLs). Resistant clones were subjected to transposon mutagenesis to identify genes conferring resistance followed by sequencing of clone inserts.

Class 1 integron prevalence varied by $>3$ orders of magnitude between soils and soil amendments sampled, persistence of these elements was also elucidated. Several novel ESBLs were discovered, with the greatest diversity present in dewatered and limed sewage sludge, including a gene showing similarity to Klebsiella OKP-A enzymes, which was adjacent to ISPme1, an insertion sequence able to mobilise adjacent segments of DNA and encoding a promoter. Several metallo $\beta$-lactamases were also found in DNA extracted from sewage sludge and sludge amended soils. Some genes conferred reduced susceptibility rather than clinical resistance, a phenomenon displayed by the progenitors of the clinically significant $\text{bla}_{\text{CTX-M}}$ ESBLs in their original genetic contexts in Kluyvera sp. Selection experiments are currently being undertaken to screen for mutations conferring high level resistance.

These data illustrate that human activity has a significant impact on resistance gene ecology and of associated genetic elements. On the basis of our data, each ton of sewage sludge contains $1\times10^{13}$ bacteria carrying class 1 integrons, which equates to $1\times10^{16}$ bacteria carrying mobile genetic elements capable of conferring antibiotic resistance being added to United Kingdom soil each year.
Gram-negative Gammaproteobacteria of the species Morganella morganii are found in the intestines of humans and several animals. Morganella morganii is associated with a broad range of infections of humans, reptiles, seals and other mammals. In healthy medicinal leeches, Morganella morganii related 16S rRNA gene sequences were determined in 16S rRNA gene clone libraries generated from the intraluminal fluid (ILF) and the intestine beside the two predominating symbiotic bacteria Aeromonas veronii and Rikenella-like bacteria. So far, the relative abundance and role of Morganella morganii in medicinal leeches has not been investigated in detail.

We aimed to investigate the abundance of Morganella morganii in the medicinal leech Hirudo verbana in detail by the use of cultivation-dependent and cultivation-independent molecular methods. Thirteen Morganella morganii related strains were isolated from the intestine and skin of diseased or dead medicinal leeches, which were characterized in detail genotypically and phenotypically in comparison to the type strain of the Morganella morganii subsp. morganii. Genomic fingerprinting methods including different rep- and RAPD-PCRs as well as multilocus sequence analysis (MLSA) were used for genotypic differentiation and substrate utilization pattern were investigated for phenotypic differentiation. In parallel, cultivation independent analysis including 16S rRNA gene based real-time PCR and denaturing gradient gel electrophoresis (DGGE) were used to determine the occurrence and relative abundance of Morganella morganii in a broad range of samples from medicinal leeches including skin and intestine samples from medicinal leeches diseased on a "red-venter-disease" and from dead leeches with unknown cause of death.

So far, a correlation with the abundance of Morganella morganii and the disease of leeches could not be found. Currently running cultivation independent molecular comparison of the abundance of Morganella morganii will give use further evidence about the occurrence and role of Morganella morganii in diseased and healthy medicinal leeches.

Dental caries in very young children is common, and may be severe and result in serious infection. Dental caries results from acid production by members of the complex biofilm community specific to the human tooth surface. Streptococcus mutans is the most common acid producer in caries initiation, but many other species appear to play a role in caries. Understanding functional redundancy and resilience in caries-associated communities will be important for developing biologic interventions. The purpose of the present study was to comprehensively examine bacterial communities associated with childhood caries and health using 16S rRNA analysis. Plaque samples were collected from 30 children under 6 years old with established severe caries in the primary dentition and 30 healthy controls. Samples were collected from all stages of caries: intact enamel, white spots, the surface of cavitated lesions, and dentin. 16S rRNA genes were amplified and sequenced using 454 GS FLX Titanium pyrosequencing. Sequences were identified by BLAST against CORE, an oral-specific database. Diversity indices and relative levels of phyla, genera and species for the progressive stages of caries were analyzed using linear mixed effects models. Community shifts were analyzed using non-metric multidimensional scaling based on Bray-Curtis Dissimilarity and cluster analysis. Community shifts were observed at all phylogenetic levels as caries stage advanced. Species diversity decreased and 74 species declined or were lost (p≤0.05 after FDR). Nineteen species, including Streptococcus mutans, Streptococcus salivarius, Veillonella and Lactobacillus, were associated with caries (p≤0.05 after FDR). Caries communities were heterogeneous, and S. salivarius appeared to be the primary acid producer in several subjects with little or no S. mutans or Lactobacillus. In addition, Veillonella levels were correlated with total levels of acid-producing species. Community diversity declined as caries advanced, raising questions about the resilience of oral communities and restoration of healthy ecosystems once caries is established. Many acid-producing species were associated with caries, suggesting functional redundancy, and S. salivarius was identified as a common alternative caries pathogen in children. In addition, levels of lactate producers and metabolizers were highly correlated.
374A  Adaption of bacteria to ameliorate the metabolic burden of carrying antibiotic resistance genes (a systems bioecology modeling analysis)
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The spread and persistence of antibiotic resistance is an important problem. It is unclear what leads to the persistence of resistance after treatment is discontinued, or the relatively high abundance of resistant bacteria in the ambient environment, where the antibiotic concentration is generally several orders of magnitude below the minimum inhibitory concentrations (MICs). Antibiotic resistance exerts a metabolic cost on bacteria and a fitness disadvantage in the absence of antibiotics which should lead to a decrease in resistance. One hypothesis is co-selection with other antibiotics or metal resistance. Alternatively, several studies have shown that bacteria can evolve to eliminate this cost. Escherichia coli can adapt to plasmid-encoded tetracycline resistance by a chromosome mutation, which requires an intact tetracycline resistance gene on the plasmid. The Tet efflux pump can mediate potassium uptake. Here the hypothesis that Tet replaces the endogenous K⁺ uptake system Trk is explored using a multi-level modeling approach that combines systems biology and systems ecology (systems bioecology). At the cellular level, the model explicitly resolves relevant processes (for example metabolism, K⁺ uptake) and the growth rate emerges. At the population level, the model simulates individual bacteria agents in competition for nutrients and the fitness emerges. The general behavior of the model is consistent with observations from the literature (for example growth rate, K⁺ limitation). In competition experiments without tetracycline, the model correctly predicts the fitness advantage of naive susceptible over naive resistant, evolved resistant over naive resistant and evolved resistant over evolved susceptible strains. Trk takes up about ten times the K⁺ required, which costs energy. Tet takes up less K⁺, which is more efficient and leads to the evolution of the Trk mutant. The evolved Trk mutant relies on Tet to take up sufficient K⁺, and thus carrying the plasmid is advantageous even in the absence of the antibiotic. The model application shows that the observed adaption is quantitatively consistent with a mutation in the Trk system. This mechanism may be one reason why the resistance is not reduced when antibiotic use or discharge is stopped, an important implications for the ecology of antibiotic resistance. We integrate this mechanism into an existing model of tetracycline in the Poudre River to explore the role of this mechanism in the ambient environment.

374B  The biotic reservoirs of Vibrio cholerae at geothermal sites at the Icelandic coast
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Vibrio cholerae was first discovered at the coast of Iceland in 2006 at isolated spots where hot water flows into the ocean, either from natural hot springs situated at the tidal zone or overflow from geothermal heating utilities, and mixes with the sea water. Cold sea water surrounds the places and the influence of the warm water is most often negligible at high tide, resulting in temperatures far below the growth temperature of V. cholerae.

The aim of the study is to determine the biotic reservoirs of V. cholerae at four representative geothermal locations at the Icelandic coast, examine the dissemination of V. cholerae from the same geothermal points and to map the environmental conditions in their habitats.

Sampling was performed at low tide at the following locations: 1) Ægissíða, Reykjavík, with geothermal 30°C fresh water outlet in the foreshore. 2) Skarðshver, a hot spring, 70-80 °C, in N- Iceland. The outlet and spring at Ægissíða and Skarðshver emerge at low tide but are covered at high tide. 3) Berserkseyri, W- Iceland, a 78°C hot spring situated ca 1 m above the tidemark. 4) Reykjanesvíkjun, SW-Iceland, a geothermal power station, where hot underground seawater, 60°C, is pumped up at magnitude 4000 l/sec, warming the surrounding sea water even at high tide. The intertidal zones of locations 1-3 are rich in vegetation, but at Reykjanesvíkjun the vegetation is poor, with large rocks and lava dominating the surroundings.

Sea samples were filtered through 0.2 μm pore size membrane filters that were incubated in APW. Macro-organisms were washed three times with sterile NaPBS solution and homogenized before incubation. Sediment samples were washed with sterile 200 ml NaPBS and macro-organisms separated by filtering through a sieve with 90 μm pore size. All samples were incubated in APW at
37°C followed by cultivation on TCBS agar. Green and yellow colonies were isolated on TS伯 agar and analyzed by biochemical tests and PCR.

All samples that were collected at Reykjanesvirkjun on three occasions were negative for V. cholerae. At the three other sampling sites influenced by the hot water, positive sea samples were obtained at all sampling occasions at 15° to 41°C. Macroscopic organisms positive for V. cholerae were borrowing lugworm, amphipods, kelp, rough periwinkle and mussels. Sixty-one % of sediment samples collected at 4-17°C were positive. Sea water samples collected 400 m up to 4 km from the three locations were positive, but a sample collected at 10 km distance from Skarðshver was negative.

The results indicate that harsh environment with no or poor fauna or flora is not a favorable habitat for V. cholerae despite a favorable sea temperature, and cultivable V. cholerae is able to reside in different organisms at temperatures fluctuating far below their minimum growth temperature.

375A Factors influencing colonization of spinach and corn salad plants by the foodborne pathogens Listeria monocytogenes and Salmonella enterica
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In the last years a growing number of outbreaks of foodborne diseases that were linked to consumption of produce (fresh vegetables) were reported. Most recent examples are an outbreak of pathogenic E. coli O104:H4 in in Germany 2011, that was associated with the consumption of contaminated seed-sprouts, and an outbreak of listeriosis in the USA 2011, that was associated with the consumption of contaminated cantaloupe. A possible source of plant contamination is the use of organic fertilizer as most of these bacteria, like Salmonella enterica, Staphylococcus aureus, Campylobacter jejuni, Listeria monocytogenes or pathogenic Escherichia coli strains, are found to be fecal contaminants. In the course of a large sampling campaign of organic fertilizers in several European countries those bacterial strains were detected frequently in slurry and manure as well as in rare cases on produce fertilized with organic fertilizers using enrichment cultures and PCR-based detection methods.

Therefore, factors that may influence colonization of human pathogenic bacteria on salad and vegetable plants, e.g. initial bacterial load, bacterial species or serovariation, plant species and organic fertilizer used, were analyzed in the present study. Therefore dilution series of two different Listeria monocytogenes serovarations and one Salmonella enterica strain were directly inoculated on corn salad or spinach seedlings grown in an axenic system or spiked in slurry or manure used as fertilizers. The plants were grown under controlled conditions in a phytochamber. After sampling, root and shoot of the plants were separated and washed. Not only the plant parts but also the washing liquid was analyzed using enrichment culture, specific PCR and fluorescence in situ hybridization (FISH) in order to test success of a simple washing step in minimizing risk of contamination.

FISH analysis revealed distinct colonization sites for the different bacterial species and serovarations also in dependence of the plant species. In general, S. enterica was able to colonize plant at lower inoculation and spiking doses compared to the two L. monocytogenes serovarations. Additionally, we observed that plant colonization was affected by the type of fertilizer used. Very low numbers of contaminated plants where detected when those were fertilized with spiked manure, even at high spiking doses. In contrast, considerably higher numbers of contaminated plants were detected when spiked slurry was used as fertilizer. Analysis of the washing liquid showed that a complete removal of the contamination was only possible in rare cases, in which plants were inoculated with a low number of bacteria.

The presented results lead to the conclusion that besides initial bacterial load in the organic fertilizer other factors like bacterial species, plant species and manure type used have an influence on colonization of fresh produce with human pathogenic bacteria. By washing the plants bacterial load was reduced but only in rare cases completely removed from the plant surface. Therefore manure instead of slurry should preferably be used in organic vegetable production in order to minimize the risk of produce contamination.
376A Can beneficial bacteria in natural prey items be transferred to lobster larvae? An onboard feeding experiment using aquaculture-reared larvae

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The ornate spiny lobster, *Panulirus ornatus*, is a high-value aquaculture candidate but commercial hatchery production is currently prevented by nutritional deficits and disease outbreaks during their four-month long pelagic larval phase. We have previously demonstrated that wild lobster larvae (phyllosomas) and their natural prey items such as arrow worms (*Chaetognatha*) and salps (*Salpida*) are good sources for isolating bacteria antagonistic to the highly virulent phyllosoma hatchery pathogen *Vibrio owensii* DY05*. Here, we present 16S rRNA gene tag pyrosequencing data from wild phyllosomas and from an onboard feeding experiment where aquaculture-reared phyllosomas were fed natural prey items for five nights to investigate whether bacterial communities associated with prey items could be transferred to and establish in mid-stage larvae. Wild phyllosomas harboured the most diverse bacterial community with respect to both species richness and evenness, followed by the arrow worms. The bacterial community of all samples were dominated by *Proteobacteria*. In addition, cyanobacteria were detected in all wild-caught zooplankton samples and *Flavobacteriales* were detected at low levels in most samples. Multivariate analysis (RDA) demonstrated that wild phyllosomas clustered with the arrow worms, primarily driven by their high relative abundance (>43%) of sequence reads affiliated with the genus *Endozoicomonas* (order *Oceanospirillales*, class *Gammaproteobacteria*). This genus has previously been detected in corals, sponges, sea slugs, sea cucumbers, ascidians and bivalves, but to the best of our knowledge has never been reported in crustaceans. All samples had a high relative abundance of sequence reads representing *Rhodobacterales* (class *Alphaproteobacteria*), but they were particularly abundant for phyllosomas from the feeding experiment (>22%). Importantly, phyllosomas from the feeding experiment could not be separated based on feeding regime and there was no evidence for transfer of genera such as *Pseudoalteromonas* and *Vibrio*, which include members antagonistic to *V. owensii* DY05*. This demonstrated that the bacterial community associated with prey items was not efficiently transferred and established in mid-stage phyllosomas during this short-term experiment. On the other hand, the bacterial community of mid-stage wild phyllosomas clustered with that of a known prey item, arrow worms. This could be because both animals independently harboured similar bacterial communities, because remnants of transparent arrow worms were present in the wild phyllosoma gut at the time of sampling, or because long-term feeding may indeed affect the bacterial community associated with wild phyllosomas. The results indicate that beneficial bacteria (probiotics) can likely be transferred to aquaculture-reared phyllosomas via formulated feeds, though the use of supplemented feed should start as early as possible and may have to be continuous.

377A Occurrence of multidrug-resistant *Serratia* spp. in the Lake Kasumigaura drainage basin, Japan

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The extensive use of antibiotics in medicine, animal farming, and agriculture has resulted in the frequent emergence of drug-resistant bacteria and the spread of resistant gene in not only human clinical but also natural environments. Agricultural activities including livestock farming are responsible for the water pollution in the Kasumigaura Lake, the second biggest lake in Japan, near the suburb of Tokyo and therefore considered highly for the increase in antibiotic potential in the lake ecosystem. In this study, the bacterial population resistant to tetracycline (TC), widely used for animal production, in the Kasumigaura Lake basin was investigated. Five sampling sites at 4 streams inflow to the lake (CP-1 to CP-4) and at lakeside (CP5) were established in the lake drainage basin and water samples were collected in every month from April, 2010 to February, 2011. Total microscopic counts stained with DAPI (4′,6-diamidino-2-phenylindole) and culturable counts on nutrient broth ranged from 6.7×10² to 2.8×10⁹ cells mL⁻¹ and from 1.9×10³ to 2.9×10⁶ CFU mL⁻¹, respectively. Culturable bacterial populations resistant to 30, 60, and 120 µg TC mL⁻¹ accounted for 5.8, 1.5, and 0.8% of culturable bacterial counts, respectively. The percentage of TC-resistant bacteria increased in July to September and decreased in November. Eighty-eight TC-resistant bacteria were isolated from water samples taken in May (55 isolates) and October (33 isolates) and analyzed for their 16S rRNA gene sequences. A half of the isolates were identified as the genus *Serratia* including *Serratia marcescens*,...
which were recovered from all the sites. The other isolates belonged to Chryseobacterium, Bucillus, Burkholderia, Providencia, and Sphingobacterium. The 37 Serratia isolates were further examined to the susceptibility to cefotaxime (CTX) and meropenem (MEM). The 17 isolates were resistant to both CTX and MEM, and the 13 isolates were resistant to CTX. The two drugs (CTX and MEM)-resistance found in the 17 isolates imply that they possessed metallo-beta-lactamase. Our results suggested that multidrug-resistant Serratia spp. prevailed in the lake basin environments, probably caused by the use of antibiotics in animal production around the lake. Further studies including analysis of multidrug-resistant genes in the isolates are now in progress in our laboratory.

377B Norlichexanthone modulate S. aureus virulence gene expression via agr and sae
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Staphylococcus aureus is a human and animal pathogen and a major cause of nosocomial infections. As infections with drug resistant S. aureus are of serious concern, new treatment strategies including modulation of virulence gene expression are being explored. Previously we identified norlichexanthone produced by fungi and lichens as a compound that potentially interferes with the agr quorum sensing system in NCTC 8325-4. Here we confirm that also in the community-associated USA300 norlichexanthone inhibits transcription of hla encoding a-hemolysin and mali, the effector of the agr, while increasing expression of spa encoding Protein A. The decreased toxin expression in USA300 was accompanied by reduced toxicity towards human neutrophils and reduced biofilm formation. As the latter observation suggests that other regulator regulatory systems than agr may be affected we performed transcriptome analysis in the presence of norlichexanthone. The result indicates that norlichexanthone represses expression of genes controlled by the transcriptional regulator, Sae. Thus our data suggest that norlichexanthone modulates virulence gene expression via several virulence regulators. This notion was supported by the observation that chlorinated analogs of norlichexanthone both reduced hla and mali expression without increasing spa expression. Thus this group of compounds may have potential as modulators of virulence gene expression in S. aureus.

378A The virulence factors in environmental Escherichia coli
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Although research has increasingly focused on the pathogenesis of Escherichia coli infections we have little knowledge about the reservoir of these bacteria. Ewers et al. (2009) supporting the hypothesis that not the ecchabat but the phylogeny of Escherichia coli strains determines virulence. These data provide strong evidence for an avian intestinal reservoir hypothesis which could be used to develop various enteric or extraintestinal infections. These strains pose a zoonotic risk because either they could be transferred directly from birds to humans or they could serve as a genetic pool for ExPEC strains. There is a substantial overlap between the phylogroups and virulence factors of Escherichia coli from human urinary infections and Escherichia coli associated with avian colibacillosis (Rodriguez-Siek et al. 2005). Our previous study (Drugdova et al. 2010) revealed that the virulence genes iutA, iss, cvaC, tsh and papC were detected significantly more often amongst meat poultry isolates than in faecal strains. The aim of this study was PCR detection of virulence factors papC, iutA, iss, cvaC, kpsII, tsh (Delicato et al. 2003), ibeA (Germon et al. 2005), integron 1 (Mazel et al. 2000) and transposon 3 (Weill et al. 2004) in phenotype ESBL positive environmental (animal waste water) Escherichia coli with comparison of human uropathogenic Escherichia coli. There was clear correlation between “chu” positivity (pathogen group B2), iutA and kpsII presence in the human urinary strains. However only one third of environmental strains belonged to the pathogen groups B2 and D. One pathogen (B2) environmental isolate E.coli ML 45 contained ibeA also with papC, iss, kpsII, integron 1, transposon 3 and cit group genes (Perez-Perez et al. 2002). Maldi tof protein analysis revealed similarity of ML45 with human UPEC. Majority environmental strains containing integron 1, belonged to the A phylogenetic group of commensals with two or three virulence factors, however two strains were without virulence factors. On the other hand occurrence of ibeA gene was detected only in B2 pathogens. Results shows that some environmental Escherichia coli with virulence factors, mobile elements and ESBLs could be the source for the human population, also.
379A  Investigating the microbiome of the bovine uterus in relation to endometritis, a costly disease for dairy farmers
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Endometritis is inflammation of the inner lining of the uterus, affecting up to 20% of the dairy cows after calving in Denmark. The disease causes reduced pregnancy rates, which often leads to culling of the cows and is costly for the farmer. Until now, investigations of which pathogens may cause the disease have been based on microbiological culturing, and no conclusive evidence has been found. Only a fraction of the bacterial flora is cultivable, and therefore more than 90% of the uterine microbiome has not been characterised. With incomplete knowledge of the pathogens, treatment is performed without an option for choosing the best suited antimicrobial agent, which may lead to unnecessary antibiotic resistance development. The present study is based on 16S rRNA PCR, which in combination with 454 next generation sequencing allows phylogenetic identification of the bacteria present in the sample. Not being limited to bacteria that are suited to growth under laboratory conditions, this study promises a more comprehensive insight into the microbiome of the dairy cow uterus than has previously been offered.

Cows (n=40) on a Danish dairy herd were randomly selected on the basis of a uterine score indicating that the cows had uterine pathology. Uterine fluid was aspirated and if necessary the uterus was flushed with 30 ml sterile saline solution in order to retrieve uterine material. The fluid was placed in RNAlater. An endometrial biopsy was retrieved and the tissue placed in RNAlater. The cows were sampled on days 5-11 (week 1), days 26-32 (week 4), and on days 47-53 (week 7). This sampling schedule provided an opportunity to follow the development of any infection, and the combination of biopsy and uterine flush samples offered insights into whether tissue-invasive bacteria were present. The DNA was extracted with the Maxwell 16 LEV Blood kit (Promega), the 16S rRNA PCR was performed with primers targeting the V2 region, and the 454 next generation sequencing was performed by GATC Biotech.

Previous papers based on culturing of endometrial swabs or biopsies point to Escherichia coli, Trueperella (Archanobacterium) pyogenes, and Fusobacterium necrophorum as the most likely pathogens, although some of them also seem to be present in healthy animals. We expect to find these bacteria in the samples from the diseased animals, and perhaps the detailed data from the sequencing will also reveal hitherto undiscovered pathogens.

380A  Human papillomavirus, cytomegalovirus and chlamydia trachomatis infections in cervical cancer patients from tiruchirapalli, tamilnadu
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Human papillomavirus (HPV), human cytomegalovirus (CMV) and Chlamydia trachomatis infection are the most common female genital pathogens. Infection with any one of these agents repeatedly associated with Cervical Intraepithelial Neoplasia (CIN) disease, prerequisite for cervical cancer. Apart from HPV the role of other sexually transmitted infections notably by Chlamydia trachomatis and viral infections by cytomegalovirus has been ruled out in this present investigation.

Eighty five cervical carcinoma cases and 96 women with normal cytology were included to determine the co-infections of human papillomavirus, with cytomegalovirus and C. trachomatis by PCR.

HPV and CMV viral DNA was detected in 72/85(84.7%) and 66/85(77.6%) of the cervical cancer patients respectively. Co-infection of these two viral DNA was detected in 58/72(80.5%) of the cases. In control women it was observed as 10/96(10.41%) for HPV and 37/96(38.5%) for CMV. C. trachomatis was detected in 46/85(54.1%) of the cases as a single infection and it was observed as 39/72(54.1%) for co-infection with HPV. In control women it was 30/96(31.25%) for C. trachomatis single infection and 2/10(20%) were co-infected with HPV. Risk factors for getting multiple infections was observed as the co-habiting women (OR: 14.7; 95% CI 3.22-67.5, P<0.001) having often sexual intercourse (OR: 5.25; 95% CI 0.59-45.6, P=0.0213) and having a history of increased abortion (OR: 4.25; 95% CI 0.87- 20.7, P=0.0394) was found to be significant.
In this present investigation we found that apart from HPV infection other viral, bacterial and sociodemographic variables are necessary for prerequisite for the CIN progression.

381A  A metaproteomic analysis of a human indwelling urinary catheter biofilm dominated by *Pseudomonas aeruginosa*
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Long-term catheterization of the bladder leads inevitably to a mostly asymptomatic bacteriuria. Adaptive response of opportunistic bacterial pathogens to the catheter environment often results in an efficient biofilm formation; the biofilm matrix can contain up to 5x10^9 viable cells per centimeter. So far, scientists investigated the microbial biofilm-forming community mainly by culture dependent methods and human catheter biofilms have never been analysed in situ. Thus, only little is known about the functional adaptation of the biofilm inhabitants, which inspired us to analyze a biofilm and urine from a long-term catheterized patient by a semi-quantitative metaproteomic approach (1D-PAGE --> LC-ESI-MS/MS) and, subsequently, to assign proteins to different phylogenetic and functional groups. *Pseudomonas aeruginosa* was found to be the predominant colonizer (160 out of 340 bacterial proteins have been assigned to *P. aeruginosa*), but also other bacteria belonging to the Enterobacteriales and Bacteroidales were present, indicating a multispecies biofilm. These findings were confirmed by quantitative 16S-ribosomal DNA sequencing. Functional assignments of proteins revealed that the most abundant proteins are involved in iron and nutrient uptake and in osmotic- or oxidative stress response. Also, the catheter-associated urine contains numerous secreted bacterial proteins, which are mainly involved in iron and nutrient uptake. To investigate the bladder specific expression and secretion of proteins in better detail, the major colonizer *P. aeruginosa* was isolated from the biofilm, cultured in artificial urine (AU) and in LB medium, followed by the analysis of the secretome. More than 130 proteins are secreted in both media and 100 uniquely in the AU media. Those AUM induced proteins could also be found in the urine, but not in the patient's biofilm. Interestingly, various components of the human immune system were present in the biofilm and urine metaproteome, i.e. factors of the complement system and neutrophils were found, which are known to play an important role during host defense, indicating a symptomatic bacteriuria. In conclusion, our study contributes to a better understanding of structure and functionality of urinary tract catheter biofilms and helps to unravel specific adaptation mechanisms employed by the opportunistic pathogens to adapt to the bladder environment.

382A  Comprehensive serotyping and epidemiology of human respiratory adenovirus isolated in Gyeonggi province, South Korea
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Adenoviruses are an important cause of respiratory tract infections, particularly in infants, young children, and immuno-compromised patients. In this study, we investigated the characteristics of adenoviruses isolated from outpatients with acute respiratory illness in Gyeonggi province of South Korea during 2009-2011. Adenoviruses were detected in 102 of 1,622 (6.3%) specimens by using PCR or real-time PCR with viral specific primers. 76 isolates were obtained from 102 specimens using the A549 cells. Serotypic distributions and genetic diversities of isolated adenovirus were analyzed by sequencing of hexon and fiber genes. Six different serotypes were identified, which included adenovirus serotypes 1-6. Adenovirus 3 (n=40, 52.6%) was the predominant serotype. The predominant types of adenovirus every year were serotypes 1 and 3 in 2009, serotype 3 in 2010, and serotype 5 in 2011, respectively. Adenoviruses 1, 2, 4, 5, and 6 were isolated sporadically throughout the study period. Adenovirus 3 was present both during outbreaks and in sporadic cases. The sequences of hexon and fiber genes of isolated adenoviruses showed small-scale nucleotide variations. These results indicate that adenovirus 3 played major causative agent of adenovirus outbreaks in Gyeonggi province of South Korea during 2009 - 2011. Continuous surveillance for specific serotypes of adenovirus that can cause outbreaks is important.
383A  Comparative ocular microbial communities in humans with and without blepharitis
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To compare the ocular microbial communities of humans with and without blepharitis, bacterial 16S rRNA genes of eyelash and tear samples from seven blepharitis patients and four healthy controls were amplified using barcoded primer sets and sequenced using a pyrosequencing method. Phylotypic analysis of 16S rRNA gene sequences demonstrated that eyelash and tear samples had highly diverse bacterial communities with many previously undescribed bacteria. Bacterial communities in eyelash samples from subjects with blepharitis were less diverse than those from healthy controls, while the bacterial communities of tear subjects with blepharitis were more diverse than those of healthy subjects. Statistical analyses using a UniFrac-based hierarchical tree and a principle coordinate analysis showed that the bacterial communities of tear samples from subjects with blepharitis were well clustered, regardless of individual, while the bacterial communities of all eyelash samples and healthy tear samples were not well clustered due to high interpersonal variability. Bioinformatic analysis revealed that Propionibacterium, Staphylococcus, Streptophyta, Corynebacterium, and Enhydrobacter were the common ocular bacteria. An increase of Staphylococcus, Streptophyta, Corynebacterium, and Enhydrobacter and a decrease of Propionibacterium were observed from blepharitis subjects, in terms of the relative abundances. Higher abundances of Streptophyta, Corynebacterium, and Enhydrobacter in blepharitis subjects suggest that human blepharitis might be induced by the infestations of pollens, dusts, and soil particles. These results will provide valuable information for the prevention and treatment of human blepharitis based on ocular microbial flora. This work was supported by the Next-Generation BioGreen 21 Program (No. SSAC2011- PJ008220), RDA, Korea.

383B  Effects of putatively prebiotic carbohydrates on pathogenic infections
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We have recently finalized a project, in which we have addressed effects of putatively prebiotic dietary carbohydrates on the susceptibility to infections with bacterial pathogens in animal models. The objective of the study was based on the pre-existing knowledge that (i) prebiotics are defined by their ability to increase the level of beneficial gut bacteria and (ii) beneficial gut bacteria are shown to counteract pathogenic infections with for example Salmonella. Thus, we hypothesized that ingestion of prebiotic carbohydrates would have a preventive effect against selected pathogenic bacteria.

Effects on Salmonella enteritica serovar Typhimurium infection were addressed in a mouse-model, while effects on infection with Listeria monocytogenes were investigated in guinea pigs because these animals carry a homologue to the human epithelial receptor for Listeria. Pathogenic counts in faeces, intestine and organs of the animals were measured. In the case of Salmonella, also immunological responses were measured. Additionally, the effect of prebiotic consumption on the intestinal microbiota of the Salmonella infected mice was assessed by Denaturing Gradient Gel Electrophoresis (DGGE) and quantitative Real-Time PCR (qPCR). In the case of Listeria, effects of prebiotic treatment on infectivity in Caco-2 cells were addressed.

In contrast to what we expected, none out of seven tested putatively prebiotic carbohydrates had preventive effects against Salmonella infection. Mice fed with fructo-oligosaccharides (FOS) or xylo-oligosaccharides (XOS) had significantly higher (P < 0.05) numbers of Salmonella in liver, spleen and lymph-nodes when compared to mice fed with a cornstarch-based control diet. Also levels of the acute-phase proteins were increased in these animals. DGGE confirmed that consumption of FOS and XOS did indeed affect the intestinal microbiota, and qPCR demonstrated an increase in the abundance of the Bacteroidetes phylum, the Bacteroides fragilis group, and of Bifidobacterium in mice fed FOS or XOS.

In guinea-pigs challenged with Listeria, we found that some dietary carbohydrates prevented the infection, while others increased its severity. In general, we found that oligosaccharide components (XOS and Galacto-oligosaccharides, GOS) improved the resistance to Listeria infection.
Our studies reveal that certain dietary carbohydrate sources may influence translocation of pathogenic bacteria from the intestinal environment to internal organs. By comparing Gram-negative and Gram-positive bacterial infections, it was elucidated that the mechanisms for these effects are likely to be very complex and to differ depending of the type of infection, as illustrated by XOS, which increased the severity of Salmonella infection in mice, but reduced the translocation of Listeria to internal organs in guinea-pigs. We find that our observations open a number of new questions related to the interaction between prebiotics, pathogens, and the intestinal environment.

384A Influence of abiotic and biotic soil characteristics on Listeria monocytogenes survival in the environment
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Listeria monocytogenes is a food born pathogen responsible of the potentially fatal disease listeriosis. Initial contamination of plants vegetables and fruits may occur in cropping systems and originate from soil (that may act as a reservoir), water or organic amendments. The aim of this study was to identify major biotic and abiotic soils characteristics which determine L. monocytogenes survival in soil. Survival of L. monocytogenes in a set of 100 soil microcosms representative of land use and pedology of French soils was evaluated. These soils were thoroughly characterized including chemical properties (pH, ionic content), texture (clay, sand and silt content), land use, and spatial localization.

A sub-set of 9 Gamma-radiation sterilized soils was used to investigate whether soil microflora may affect L. monocytogenes survival. Microcosms were inoculated with $10^6$ L. monocytogenes per gram of soil. L. monocytogenes populations were monitored after 7, 14 and 84 days of incubation at 20°C by plate-counts on selective PALCAM media. L. monocytogenes was able to persist in most of the tested soils but survival rates were strongly variable. A short term and a long term survival of L. monocytogenes were reported in 71% and 21% of tested soils, respectively. In 8% of soils, L. monocytogenes was either absent or below detection limits as soon as 7 days after inoculation. This suggests that L. monocytogenes survival depends on abiotic and/or biotic factors. Statistical analysis evidenced that land use and spatial localization of soils did not significantly affect L. monocytogenes survival. Variance partitioning demonstrated that 60% of the variations of survival rates at 7 and 14 days were explained by soil chemical properties. Basic cation saturation ratio was the major soil chemical characteristic explaining short-term survival. Cationic exchange capacity and exchangeable calcium further explained survival of L. monocytogenes at 7 days and 14 days respectively. Long-term survival was driven mainly by soil texture (11% of the variance), especially clay content. Survival of L. monocytogenes in sterile soils differed. Indeed, 4 sterilised soils supported growth of L. monocytogenes while the rate of decrease was lower in the other sterilised soils than when microflora was active. This result points out the critical role played by both the soil microflora and the soil physico-chemical properties in determining the survival rate of L. monocytogenes.

385A Identifying genomic and metabolic features that can underlie early successional and opportunistic lifestyles of human gut symbionts
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We lack deep understanding of genetic and metabolic attributes specializing microbial consortia for initial and subsequent waves of colonization of our body habitats. Here we show that phylogenetically interspersed bacteria in Clostridium cluster XIVa, an abundant group of bacteria in the adult human gut also known as the Clostridium coccoides or Eubacterium rectale group, contains species that have evolved distribution patterns consistent with either early successional or stable gut communities. The species that specialize to the infant gut are more likely to associate with systemic infections and can reach high abundances in individuals with Inflammatory Bowel Disease, indicating that a subset of the microbiota that have adapted to pioneer/opportunistic lifestyles may do well in both early development and with disease. We identified genes likely selected during adaptation to pioneer/opportunistic lifestyles as those for which early succession association and not phylogenetic relationships explain genomic abundance. These genes reveal potential mechanisms by which opportunistic gut bacteria
tolerate osmotic and oxidative stress and potentially important aspects of their metabolism. These
genes may not only be biomarkers of properties associated with adaptation to early succession and
disturbance, but also leads for developing therapies aimed at promoting re-establishment of stable gut
communities following physiologic or pathologic disturbances.

386A  Is *Faecalibacterium prausnitzii* a good indicator of healthy intestinal mucosa-
associated microbiota?
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*Faecalibacterium prausnitzii* is one of the most abundant anaerobic commensal bacterial species that
inhabits the human large intestine, suggested to play a key role in maintaining a healthy gut. There is
an increasing interest concerning this species, since it has been reported to be reduced in the elderly
and under certain intestinal disorders as inflammatory bowel diseases. However, to date, no
consistent study has been conducted regarding its abundance in other bowel diseases.

The aim of this work was to determine to which extent mucosa-associated *F. prausnitzii* abundance is
compromised in other intestinal disorders apart from Crohn’s disease and to assess if this bacterium
could be regarded as a general indicator of a healthy gut microbiota.

Mucosa-associated *F. prausnitzii* quantity was determined by using a new real-time PCR assay in 40
control subjects and 125 age and sex matched patients suffering from bowel diseases of different
nature: Crohn’s disease (n=58), ulcerative colitis (n=29), inflammatory bowel syndrome (n=7),
determinate colitis (n=3) and colorectal cancer (n=28). When possible, biopsies from different zones
of the gastrointestinal tract were analysed to check for changes along the gut. Results were
normalized by referring to human cells to correct sample size variability. Statistical analyses
comparing patients’ diagnostics and considering clinically relevant data within each subgroup of
subjects, were performed using Mann-Whitney U tests with SPSS v15.0.1 software.

*F. prausnitzii* abundance was decreased approximately threefold in Crohn’s disease patients
compared to control subjects, irrespectively of the intestinal zone sampled (p=0.006). Within Crohn’s
disease patients, differences among localizations were observed. Patients with ileal involvement
showed lower *F. prausnitzii* numbers than those with colonic disease, which is in agreement with
previously reported data. No significant differences were observed when medication, smoking habit,
activity and disease duration were taken into account. Interestingly, *F. prausnitzii* abundance was four
times greater in individuals suffering from ulcerative colitis than in those with Crohn’s disease
(p=0.001), regardless of disease evolution, number of previous outbreaks or extension of the disease.
Also colorectal cancer patients showed decreased levels of *F. prausnitzii* than control subjects
(p=0.039). No significant differences were found in patients with inflammatory bowel syndrome or
determinate colitis, when compared to the control group.

This study provides evidence that *F. prausnitzii* is selectively depleted in Crohn’s disease among
inflammatory bowel diseases and suggests that this bacterium could not be regarded as a general
indicator of healthy gut status. Since ulcerative colitis patients harbour increased levels of this species,
*F. prausnitzii* abundance can be used as a quantitative indicator to distinguish between these diseases
in adults. Our data reinforces the hypothesis of a particular dysbiosis implicated in ileal Crohn’s
disease pathogenesis and gives clues for future therapies by restoring this bacterial population in
patient with this specific intestinal condition.

387A  Fungi and bacteria in nasal lavage from greenhouse workers as affected by
environmental exposure
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The nose is the first region of the respiratory tract in contact with airborne microorganisms. Exposure
to fungi, (1→3)-β-D-glucan (β-glucan) and bacteria is associated with development of different
respiratory symptoms. The inhalation and sedimentation of microorganisms in the respiratory tract depend on the aerodynamic diameter of the microbial particle. Damage to the pulmonary system is associated with particle deposition in the airways. Airborne fungi and bacteria can be found in different size fractions and are often present as or associated with particles between 2 μm and 6 μm.

Greenhouse workers can be exposed to high concentrations of airborne fungi and bacteria from the environment but the sedimentation of airborne microorganisms in the nasal cavity is not described. Presence of fungi or bacteria has sometimes been measured in nasal lavage (NAL) but not quantitatively and not together with environmental exposure. In this study we have addressed the following question: Are concentrations of fungi, β-glucan and bacteria in NAL affected by environmental exposure, gender, time of the work day and concurrent rhinitis symptoms.

We have measured the concentrations of fungi, β-glucan and bacteria in 164 NAL samples from greenhouse workers taken Monday morning and Thursday at noon in 2010 and 2011. During the same week we measured personal exposure to inhalable bioaerosols during whole working days.

The concentrations of fungi, β-glucan and bacteria in NAL were significantly higher Thursday at noon than Monday morning. The concentrations of fungi and β-glucan, but not bacteria, in NAL from men were significantly affected by the personal exposures. The concentrations of fungi, β-glucan and bacteria were significantly higher in NAL from male workers than in NAL from female workers. Rhinitis symptoms had a significant effect on concentration of fungi in NAL with the lowest concentrations for workers with rhinitis symptoms.

A significant correlation was found between β-glucan per fungal spore in NAL versus in the exposure. However the concentration of β-glucan per fungal spore was higher in NAL than in the exposure.

Men and women worked in the same environment with somewhat similar tasks. The concentration of fungi and β-glucan in NAL from men were highly affected by their exposure from the environment while concentrations in women's NAL were unaffected. Furthermore, the concentration of bacteria in NAL was only affected by the time of the week for men. The reasons to these differences between men and women may partly be caused by differences in breathing pattern and hygiene. The higher amount of β-glucan per fungal spore in the NAL than in the air indicates that some of the smaller fungal particles have passed the nasal cavity.

In conclusion the concentrations of fungi and β-glucan, but not bacteria, in NAL from men were significantly affected by the environmental exposure. Concurrent rhinitis symptoms significantly affected the concentration of fungi in NAL of men.

Otitis media (middle ear infection) is endemic in many remote Indigenous Australian communities. Cross-sectional studies identify tympanic membrane perforation and chronic suppurative otitis media (CSOM) in up to 24% of young Indigenous children in the Northern Territory – a rate approximately 6 times that recommended by the World Health Organization as requiring urgent public health attention. Standard antibiotic therapies for acute otitis media have had limited effect in reducing progression to CSOM in this population. The dense, polymicrobial bacteriology associated with otitis media in this population likely contributes to the high clinical failure rates.

The aim of this pilot study was to use T-RFLP and PhyloChip™ analyses to characterise bacterial communities in nasopharyngeal and ear discharge swabs from Indigenous Australian children with acute otitis media with perforation (AOMwiP). Paired baseline swabs from 10 children with AOMwiP were randomly selected from a clinical trial of azithromycin versus amoxycillin treatment. AOMwiP was
defined as the presence of a perforation (no more than 2% of the tympanic membrane) for less than 6 weeks. The children had not been prescribed antibiotics for at least 7 days prior to specimen collection. Nasopharyngeal and ear discharge swabs were characterised by T-RFLP using 16S rDNA primers 27F and 1113R, and restriction enzymes CfoI and NlaIV. T-RFLP profiles were compared using non-metric multi-dimensional scaling and hierarchical group average clustering analysis. PhyloChip™ analysis was used to further characterise five of the ear discharge swabs (performed as a commercial service by Second Genome).

2-12 peaks were present in each T-RFLP profile. Significant site-specific clustering was observed for nasopharyngeal and ear discharge profiles (Analysis of Similarities (ANOSIM): global R 0.62; P=0.001). In contrast, no clustering was observed between paired nasopharyngeal and ear discharge profiles from individual children. PhyloChip™ analysis detected 29-47 phyla in each ear discharge swab (Bacteria and Archaea). 12 phyla and 26 families consistent with otitis media pathogens, skin flora, gut flora, anaerobes and environmental bacteria were common to all five ear discharge swabs. In four ear discharge swabs, highest richness was observed for Pseudomonadales and Enterobacteriales, possibly indicating progression to CSOM. The remaining swab had over 10-fold fewer OTUs detected and increased Lactobacillales richness, specifically Streptococcaceae and Staphylococcaceae.

This study has demonstrated that complex polymicrobial communities are present in nasopharyngeal and ear discharge swabs from Indigenous Australian children with AOMwiP. The T-RFLP data showed higher similarity between swabs from the same anatomic site than between paired swabs from individual children, suggesting the bacterial communities in the nasopharynx and ear discharge are different. Collectively, the T-RFLP and PhyloChip™ data prompt further consideration of the canal flora in relation to secondary middle ear infection following tympanic membrane perforation. Larger studies are planned to determine if changes in bacterial richness and community structure contribute to clinical progression from AOMwiP to CSOM. These data may inform application of novel strategies for prevention and treatment of CSOM.

389A Evolutionary linkage between aquatic predation-resistant bacteria and mammalian pathogenic bacteria

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There are several pathogenic bacteria that have aquatic environments as their reservoir, *Mycobacterium* ssp., *Escherichia coli* and *Francisella tularensis* are examples. Previous research shows that these bacteria can survive predation from protozoa, which is their main bacterial predator in aquatic environments. Increased eutrophication results in an increased predator:prey ratio, i.e. protozoa:bacteria ratio, which means that the selection for predator-resistant bacteria also increases. Possibly also a selection for bacterial pathogens and for mechanisms causing increased virulence would occur. These results are alarming since eutrophication has been increasing in many areas due to anthropogenic impact and is predicted to continue to increase, potentially resulting in more hostile aquatic environments. We aimed to further test the linkage between environmental survival and the ability to infect a susceptible host. This was addressed by using *Francisella tularensis holarctica* (FSC 200) and four mutants (*hfq, dsbA, pilA and pglA*) to compare intracellular survival in a murine macrophage cell line (*J774*) and the aquatic protozoa *Acanthamoeba castellanii*. The wild type is known to resist degradation and replicate within both *J774* and *A. castellanii*. The mutants were selected based on a hypothetic or previously shown role for *Ft. holarctica* virulence. Both *J774* and *A. castellanii* attach to surfaces enabling us to use similar methods for monitoring cell-associated growth of *Ft. holarctica*. Cells were seeded in a 24-well tissue plate, infected and harvested to measure the intracellular content of bacteria over time (*J774*: 48 hours and *A. castellanii*: 12 days). Intracellular content was grown on McLeod agar plates for viable count. The performances of the bacterial strains in the two model systems (macrophage and amoeba) were strikingly similar. Of the mutants, PilA was the most attenuated for growth in *A. castellanii*. PilA is a pilin protein which has been shown to be important for virulence in *F. tularensis*. Also Hfq and DsbA are important for virulence according to previously published data from a macrophage assay. Hfq is an RNA binding protein which promotes
cellular stress responses while DsbA is involved in folding of membrane or secreted proteins. The attenuated dsbA mutant was also significantly attenuated in *A. castellani*. Moreover, Hfq also seems to have impact on survival in both environments, although these effects were not as pronounced. PglA is involved in posttranslational glycosylation of pilins, for example PilA. The PglA strain was not attenuated in either J774 or *A. castellanii*, indicating that PglA has no essential role for intracellular survival. These findings reveal mechanisms in *Ft. holarctica* of importance for intracellular growth and replication in an aquatic predator and a mammalian macrophage. This strengthens the evidence of not only an environmental link but also a strong evolutionary link between the aquatic and the mammalian environment. Due to these links we suggest that eutrophication can cause increased selection for virulence mechanisms and thus pathogens in aquatic environments.

389B  Environmental monitoring of pathogens during bioremediation using massive parallel 16S rRNA gene sequencing
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Given the potential for a variety of microbial pathogens to occur in bioengineered ecosystems like bioremediation sites, an understanding of pathogen diversity and abundance is necessary for accurate assessment of microbial risk in such environment. Massive parallel sequencing technology makes sequencing deeply into rare microbial populations possible such that pathogen diversity and abundance can be explored. To test this possibility, we monitored microbial population changes during bioremediation of chlorinated ethenes using high-throughput 16S rRNA gene sequencing. 454 amplicon-pyrosequencing technology was used to produce large 16S rRNA gene libraries (average 25,404 sequences per sample, 1,422,629 sequences in total). To identify pathogens we developed a dataset containing >990 16S rRNA gene sequences of bacterial pathogens that are listed as Bio-Safety Level (BSL) 1* to 3 by Biosafety committee of Japanese Association of Bacteriology. We also prepared a 16S rRNA gene dataset of 81 phylotypes of known dechlorinating bacteria for monitoring of bioremediation processes. QIIME pipeline was used to group and classify the sequence reads, and the pipeline was also used for detecting and quantifying possible pathogens (>99% similarity threshold was set) and dechlorinating bacteria (>97% similarity threshold was set). Full-scale biostimulation experiments were conducted by supplying nutrients in two different sites where groundwater was polluted with tetrachloroethene (approximately 0.5 mg/L) and trichloroethene (approx. 0.4 mg/L). After the injection of nutrients, tetrachloroethene in the groundwater was successfully dechlorinated and completely removed in 30-40 days at the injection site. Soil and groundwater samples at different locations around the remediation sites were collected during biostimulation. Total genomic DNA was extracted from the samples and the V4 region of 16S rRNA genes were amplified and used for pyrosequencing. After the injection of nutrients, relative abundance of *Firmicutes* and *Bacteroidetes* in groundwater increased, suggesting that groundwater became anaerobic due to the injection. Alpha diversity values based on the sequence reads transiently decreased after the injection, but were recovered during the remediation process. 14 phylotypes that are close to known dechlorinating bacteria such as *Dehalococcoides* spp. were successfully detected and monitored during the bioremediation. 25 and 3 phylotypes that are close to BSL1* and BSL2 pathogens, respectively, were also detected and quantified. No possible BSL3 pathogens were detected. Most of possible BSL1* pathogens detected were those of the genera *Comamonas* and *Acinetobacter*. Relative abundance of possible BSL1* pathogens in the microbial community slightly increased right after the injection, accounting for approx. 2.8% of the total reads. However, their abundances rapidly decreased during the remediation process (<0.7% of the total reads). BSL2 pathogen-like phylotypes were found to be related with those of the genera *Escherichia*, *Mycobacterium*, and *Yersinia*; their relative abundances were always lower than 0.1% of the total reads. In conclusion, we investigated possible pathogen diversity and abundance during bioremediation based on 16S rRNA gene pyrosequencing. The analysis described here can be used to determine the diversity of possible pathogens in an environmental sample. This work provides basis for better estimating microbial risk for bioengineered ecosystems.
390A Structural Stability of a Salivary Bacterial Population against Supragingival Microbiota Shift Following Periodontal Therapy
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Supragingival plaque is permanently in contact with saliva. However, the extent to which the microbiota contributes to the salivary bacterial population remains unclear. We compared the structural shift in the salivary bacterial population with that in supragingival plaque following periodontal therapy, which seemed to affect the supragingival plaque microbiota.

Samples were collected from 19 patients with periodontitis before and after periodontal therapy (mean sample collection interval, 25.8 ± 2.6 months), and their bacterial composition was investigated using barcoded pyrosequencing analysis of the 16S rRNA gene.

Phylogenetic community analysis with the UniFrac distance metric revealed that the overall bacterial community structure of saliva is distinct from that of supragingival plaque, both pre- and post-therapy. Temporal variation following therapy in the salivary bacterial population was significantly smaller than in the plaque microbiota, and the post-therapy saliva sample was more similar to that pre-therapy from the same individual than those from other subjects. Following periodontal therapy, microbial richness and biodiversity were significantly decreased in the plaque microbiota, but not in the salivary bacterial population. The operational taxonomic units whose relative abundances changed significantly after therapy were not common to the two microbiota.

These results reveal the structural stability of salivary bacterial populations against shifts in the subgingival microbiota, suggesting that the effect of the supragingival plaque microbiota on salivary bacterial population structure is limited.

391A Evaluation of a vegetative treatment system to reduce fecal microorganisms and antibiotic resistant bacteria in beef cattle feedlot runoff
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Vegetative treatment systems designed to treat beef cattle feedlot runoff are an alternative to holding ponds and involve short-term runoff accumulation in basins and application to grass treatment areas. Field evaluations are needed to determine if pathogens, fecal indicators, and antibiotic resistant microorganisms are reduced during standard operation. Total coliforms, E. coli, Enterococcus, and STEC O157 were monitored using culture-based and molecular methods over two years in feedlot runoff and in treatment area soil samples collected to a 50 cm depth at a VTS site in central Nebraska, USA. Tetracycline- and cephalosporin-resistant bacteria were also enumerated in runoff events and from soil samples using selective media. Feedlot runoff was applied on six separate dates during the study period and soil cores (0 to 50 cm) were collected annually. Large variations (up to four orders of magnitude) in microbial runoff water quality parameters were observed between dates. Total coliforms, E coli, and Enterococcus in the feedlot runoff averaged 3.4 \times 10^5, 2.2 \times 10^5, and 3.3 \times 10^3 per mL, respectively. STEC O157 was detected in 92.5% of the feedlot runoff and in 77.4% of the samples collected at the downslope end of the treatment areas where excess feedlot runoff accumulated. Tetracycline-resistant bacteria were detected in all feedlot runoff and in all samples where excess feedlot runoff accumulated. Cephalosporin-resistance was less pervasive and was present in 22.5% of the feedlot runoff and 10% of the downslope runoff samples. To investigate the persistence of fecal microbes after effluent application, runoff generated from direct rainfall precipitation on the treatment areas was tested and found to be negative for STEC O157 and cephalosporin-resistant microorganisms. However 70.8% of the rainfall runoff samples were positive for tetracycline-resistant microorganisms. Soil depth profile samples of total coliforms, E coli, and Enterococcus from four replicate treatment areas sampled in autumn showed distinct vertical profiles. The abundance of was highest (P < 0.05) at the surface for total coliforms and Enterococcus (4926 and 2062 per gram soil, respectively) and lowest in the deepest cores (103 and 530 per gram soil, respectively). E. coli abundance was low, decreasing with depth (19.5 to 0.3 per gram soil surface to bottom). Only a single surface soil sample tested positive for STEC O157; no other depths or other sites were positive for
STECC O157. Similarly, no tetracycline or cephalosporin-resistant microorganisms were detected in any of the soil samples. There were no differences between indicator and pathogenic microbes in the soil profiles of treatment areas receiving feedlot runoff compared to the berm areas that received no feedlot runoff with the exception of higher coliform counts in the surface soils (0-5 cm and 5-10 cm) of the berm soil samples. Based upon the microbiological results, we conclude that vegetative treatment systems are a good tool to manage fecal microorganisms and antibiotic resistant microbes commonly found in beef cattle feedlot runoff.

392A Diatom-indicated characteristics of aquatic plant biofilms associated with Mycobacterium ulcerans
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Mycobacterium ulcerans is the etiological agent of Buruli ulcer, an emerging, devastating skin and bone disease. M. ulcerans occurs naturally in aquatic ecosystems, but understanding of its ecology and transmission is limited. Observations of M. ulcerans on macrophyte surfaces suggest biofilms may be a key niche, but specific habitat requirements of M. ulcerans in biofilms are unknown. Diatoms are a diverse group of eukaryotic algae common in aquatic biofilms, and are used worldwide as biological indicators of ecological condition due to their response to a wide range of environmental parameters. We hypothesized that diatoms in biofilms harboring M. ulcerans could be used to characterize the microhabitat of biofilms suitable for M. ulcerans colonization. We collected biofilms from 150 macrophytes in Ghana, Africa and used PCR-based methods to assess the presence of M. ulcerans via amplification of the enoyl reductase (ER) domain. Diatom species in each biofilm were identified, and habitat preferences of each species used to characterize microhabitat conditions. Specifically, diatoms were used to indicate the environmental conditions for organisms in biofilms with regard to pH, salinity, nitrogen uptake metabolism, oxygen requirements, trophic state, sediment tolerance, and drying tolerance. Diatom assemblages were different between lotic (flowing water) and lentic (still water) habitats, so we hypothesized that factors affecting M. ulcerans presence would be different between lotic and lentic habitats, and compared them separately. M. ulcerans DNA (ER) was identified on 43% of lotic plants and 34% of lentic plants sampled. Overall diatom assemblages were different between ER+ and ER- plants in both lotic and lentic habitats, suggesting drivers of diatom assemblages and M. ulcerans (ER) presence were similar. In lotic habitats, classification tree analysis using diatom-indicated microhabitat conditions showed that M. ulcerans was more likely to occur in low salinity biofilms, or in mildly saline biofilms with relatively higher pH. In lentic habitats, M. ulcerans was more likely to be present in biofilms containing diatoms tolerant of sedimentation, or in lower sediment biofilms in eutrophic conditions. Our data indicate that the presence of M. ulcerans in aquatic plant biofilms may be driven by environmental factors, but that relationships are complex and differ between lotic and lentic habitats. Specifically, these data suggest that water chemistry may be an important predictor of M. ulcerans in naturally unstable lotic habitats, but that land use-related factors may be key parameters driving M. ulcerans presence in lentic water habitats that are potentially less adapted to disturbance. Identifying habitat requirements of M. ulcerans and environmental changes that can promote its emergence may help identify specific areas of risk that, with further understanding and communication, could be used to reduce transmission of Buruli ulcer disease.

393A Environmental reservoirs of the footrot pathogen Dichelobacter nodosus and transmission dynamics of the disease
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Over 95% of sheep flocks in the UK have sheep with footrot, and 8–10% of sheep are affected at any one time. The disease presents with a range of severities, from mild interdigital inflammation (interdigital dermatitis), to separation of the hoof horn from the foot, all severities are associated with lameness. The causative agent, Dichelobacter nodosus is an obligate anaerobe that can survive outside the host, suggesting that the environment plays a role in disease transmission. It is possible to differentiate D. nodosus strains based on the region encoding the polymorphic proline-glycine repeat (Pgr) protein. Therefore the aim of this study was to investigate the transmission dynamics of Dichelobacter nodosus at parturition using pgr as a molecular marker.
Foot swabs were collected from ten lambs at birth, and from the same lambs and their dam 5-13 hours later; bedding samples were also collected from the lambing pen at the second timepoint. DNA was extracted from all samples using standard techniques, and both nested and quantitative PCR was used to determine the presence and load of *D. nodosus* in each sample. In *D. nodosus* positive samples, the pgr region was amplified and amplicons of varying size cloned to permit sample characterisation and further analysis.

*D. nodosus* was undetectable on lambs feet at birth, but after 5-13 hours, the pathogen was detected. There was a lower load detected on the feet of lambs compared to their dam, and ranges from $10^3$ to $10^4$ in lambs and $10^5$ to $10^6$ in ewes. Environment was found to be a reservoir as cells were detected in bedding.

These results indicate that the environment plays a key role in the transmission of the pathogen between dam and offspring from a very early age. It appears that lambs are colonised by *D. nodosus* when they are very young, and this may have implications for lambing management.

In addition work is underway to investigate *D. nodosus* survival in soil. Survival was greater at low soil matrix potential (–kPa 261), compared to high (- kPa).

**Tick microbiomes revealed by Batch Learning Self-Organizing Maps (BLSOMs)**

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Tick (Acari: Ixodida) is one of the most known blood-sucking arthropods that transmit a variety of pathogens to humans and animals, including viruses, bacteria, and protozoa. With the increasing opportunities of human/animal contact with natural foci of tick-borne infection and expanding the distributions of vector ticks due to environmental changes, the increasing number of novel tick-borne pathogens has been reported. Above all, many of the rickettsial pathogens identified as emerging pathogens in the past few decades were firstly discovered in ticks and subsequently recognized as the causative agents of animal or human diseases, indicating that an active surveillance for potential pathogens in ticks may be feasible approach to combat emerging tick-borne diseases. Given the great diversity of tick species as well as their wide geographic distribution, it is very likely that the as-yet unidentified pathogens are harboured in ticks. The aim of this study was to reveal a diversity of tick microbiomes which may contain as-yet unidentified pathogens using a metagenomic approach.

Seven tick species (both field-collected and laboratory-reared) were used in the present study. Purified bacteria-enriched fraction prepared from tick pool was subjected to DNA extraction and pyrosequencing after whole genome amplification. Resulting sequence reads were phylotyped using a Batch Learning Self-Organizing Map (BLSOM) program, which allowed phylogenetic estimation based on the similarity of oligonucleotide frequencies, and functionally annotated by BLASTX similarity searches.

We showed that each tick pool had different bacterial composition. A variety of bacteria were identified using BLSOM, including those previously associated with human and animal diseases such as genera Anaplasma, Bartonella, Borrelia, Ehrlichia, Francisella, and Rickettsia. In addition, certain tick species were revealed to possess bacteria classified into the phyla Chlamydia and Chlamydomphila as dominant taxa/populations, indicating that these Chlamydiad organisms may serve as potential tick-borne pathogens. The sequences associated with the putative pathogen-associated factors were identified from the metagenomic libraries using BLSATX-based method. Not only field-collected but also laboratory-reared ticks were revealed to have those factors, indicating that ticks may be persistently infected with potential pathogens even without a contact with their natural habitats or reservoirs. When BLSOM was applied to estimate the phylogenetic origins of those factors, many of them remained unclassified. On the other hand, the BLASTX searches against NCBI nr database revealed that most of those sequences had higher similarity with pathogenic genes of prokaryotes, suggesting that those sequences possessed their functions but lost sequence characteristics such as oligonucleotide
frequencies. One hypothesis is that those unclassified sequences were introduced through ancient horizontal gene transfer and lost their oligonucleotide frequencies in the recipient genome including those of other bacterial groups.

In conclusion, our efforts to construct a database of tick microbes may lead to the empowerment to predict emerging tick-borne diseases. A comprehensive understanding of the tick microbiomes will also be useful to understand tick biology including vector competency and interactions with pathogens.

195B  **In situ multilocus variable number of tandem repeats based, direct typing methods of *Vibrio splendidus* in oyster (*Crassostrea gigas*) haemolymph**

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Pacific oysters (*Crassostrea gigas*) are encountering increased mortality outbreaks worldwide that were first noticed in France in 2008 and then rapidly expended. Pathogens isolated from moribund oysters during these mortalities include the Oyster Herpes virus OsHV-1 (see related summary) but also the γ-proteobacterium, *Vibrio splendidus*, frequently identified in moribund oysters, especially in the oyster’s haemolymph. Recent extensive sampling programs performed by the European FP7-funded BIVALIFE consortium have allowed the establishment of a large strain collection that has been shown to belong mainly to *Vibrio splendidus* related species. Indeed *Vibrio splendidus* belongs to a polyphyletic clade and formal identification of the bacterium requires classical sequencing of different housekeeping genes and is thus long and expensive. Beside identification problems at a species level, tools are required to discriminate between strains or isolates and to investigate pathogenic strains diversity in situ without long preliminary strain enrichments. Recently using available genomic data, we developed a Multiple Loci “Variable Number of Tandem Repeats (VNTR)” Analysis (MLVA) for this clade as the basis of a cheap and rapid detection and typing method for epidemiological purposes and for the characterization of the most virulent species, biovars or strains.

Here, basing our approach on the previously selected set of 7 general and specific primer pairs (ACEFLOR) that constituted the basis for a multiplex PCR one step identification and typing method of a *V. splendidus* and *V. splendidus*-like strains, we developed an in situ direct typing method from oyster haemolymph. Oysters were artificially infected in aquaria by a chloramphenicol-resistant *V. splendidus* strain derivative and whole community DNA of healthy and artificially inoculated animals was extracted and purified using Quiagen environmental DNA extraction kits. The MLVA ACEFLOR typing could be successfully performed on contaminated samples while no signal was obtained from healthy controls. Sensitivity of the test was assessed by comparing answers of successive whole community DNA dilutions and counts on selective media opening the route to direct typing of oyster samples throughout Europe for epidemiological purposes. Field validation should be confirmed.

395A  **A bistable switch and anatomical site control Vibrio cholerae virulence gene expression in the intestine**

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Affecting 3–5 million people and causing up to 100,000–130,000 deaths per year, *Vibrio cholerae* is a severe diarrheal disease. During cholera outbreaks, an extensive amplification and transmission of the bacterium is observed. For gastrointestinal infections, the pathogen often enters an already occupied environment and needs to colonize a particular niche. It is fundamental for the pathogen to replicate during infection; deliver virulence factors at the right time and location, and to increase the transmission rate to new hosts.
Microarray and in situ single cell expression methods were used to study Vibrio cholerae growth and virulence gene expression during infection of the rabbit ligated ileal loop model of cholera. Genes encoding the toxin-coregulated pilus (TCP) and cholera toxin (CT) were powerfully expressed early in the infectious process in bacteria adjacent to epithelial surfaces. Increased growth was found to co-localize with virulence gene expression, suggesting that virulence may play a role for colonization of the host.

Significant heterogeneity in the expression of TCP and CT was observed late in the infectious process. A bistable epigenetic switch was found to be responsible for the bifurcation in virulence gene expression. This bistable switch requires the positive-feedback circuit controlling ToxT expression and formation of the CRP-cAMP complex during entry into stationary phase. Key features of this bistable switch also were demonstrated in vivo, where striking heterogeneity in tcpA expression was observed in luminal fluid in later stages of the infection. When this fluid was diluted into artificial seawater, bacterial aggregates continued to express tcpA for prolonged periods of time. The bistable control of virulence gene expression points to a mechanism that could generate a subpopulation of V. cholerae that continues to produce TCP and CT in the rice water stools of cholera patients, thereby possibly increasing the transmission rate to a new host.

395B  Bacteroides vulgatus strain PC510 attenuates expression of the NF-kB signaling pathway in HT-29 cells
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Bacteroides vulgatus is among the most commonly isolated “commensal” bacteria from the human gut. However, recent studies suggest that some strains are capable of attaching to and invading colonic epithelial cells and inducing pro-inflammatory cytokines; and B. vulgatus-specific antibodies have been identified in Crohn’s disease (CD) subjects. We have isolated a new strain of B. vulgatus (PC510) and our analysis of the draft genome sequence revealed that the strain possesses a putative lipoprotein and efflux transport system with similarity to proteins encoded by a CD gut derived metagenomic fosmid clone (52B7). This clone was identified by Blottière and colleagues at INRA and shown to activate NF-kB mediated transcription using an HT-29-based reporter cell line. Cultures of B. vulgatus strain PC510 have also now been shown to activate the regulatory cascade, but although the “type strain” of B. vulgatus ATCC 8482 also contains these genes, recent studies have shown the strain does not produce the same effects on the HT-29 NF-kB reporter cell line as does the fosmid clone, or strain PC510. This suggests that the lipoprotein and efflux system are necessary, but may not be sufficient for modulating the regulatory cascade; there may be additional structural and/or regulatory elements required that are absent or deficient in B. vulgatus ATCC 8482. Our analyses suggest that while there are localized syntenic blocks between 52B7, B. vulgatus PC510 and B. vulgatus ATCC 8482, the degree of global synteny is poor. These preliminary studies have helped delimit the genomic region that may account for the variation in immunomodulatory properties among B. vulgatus strains however, further studies are necessary to identify additional structural/regulatory elements that support the production of the immunomodulatory factor(s). We are now using a combination of RNA-seq and RT-PCR analyses to better characterize global and specific gene expression by B. vulgatus PC510, with a view to identify and understand what biotic and/or abiotic factors may affect the immunomodulatory properties of this bacterium.

396A  Bacterial and chemical contributions to aquatic environments: study of a medical center – WWTP – river continuum and a pasture land – creek - river continuum
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Massive antibiotic use and emergence of bacterial resistance associated are regarded as major threat for public health on a world-wide scale. In aquatic environments, antibiotic-resistant fecal bacteria from human origin were mainly released via effluents of wastewater treatment plants (WWTPs) whereas antibiotic-resistant fecal bacteria from animal origin were mainly released by run-offs or soil leaching. In this context, the seine-aval research program (http://seine-aval.crihan.fr/web/) carried out many
studies along the most anthropised European estuary: the Seine estuary. To better understand dynamics of the contamination by antibiotic-resistant faecal bacteria and antibiotics in aquatic environments, a multidisciplinary study have focused on a small watershed (123 km²) whose land and antibiotic uses were well assessed in partnership with hospital pharmacists, general practitioners and veterinary surgeons. The aims of this study were (i) to investigate the relationship between antibiotics and antibiotic-resistant faecal bacteria in waters along a medical center-WWTP-river continuum during a maximal antibiotic use period and (ii) to assess levels of contamination by antibiotic-resistant faecal bacteria and antibiotics according to land use and hydrological conditions along a pasture land-creek-river continuum.

The sampling strategy takes into account hydrologic and epidemiologic periods in order to study the spatial contamination of water by both antibiotics and antibiotic-resistant faecal bacteria. The detection of antibiotic compounds in waters was performed with a multiresidue chemical analysis methodology, using Solid Phase Extraction coupled with Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS), which is a powerful technique to detect low levels of 41 antibiotics. In addition to antibiotic-resistance analysis, population structures of faecal bacteria were investigated by the distribution in four main phylo-groups in E. coli (A, B1, B2, D) and diversity of enterococci species.

Along the medical center-WWTP-river continuum, amoxicillin was the most prescribed antibiotic at the medical center and by general practitioners but only the persistent antibiotics (sulfamethoxazole, ofloxacin, roxithromycin) were found in the river and consequently without relationship to the main resistances of E. coli and Enterococcus isolated in the river. Interestingly, occurrence and diversity of antibiotic-resistant faecal bacteria evolved along the continuum: E. coli carrying class 1 integrons and Enterococcus faecium of the clonal complex CC17 decreased from medical sources to the river.

Along the pasture land-creek-river continuum, antibiotic contamination was higher at the downstream of the watershed although the most persistent antibiotics (from 1ng.L⁻¹ to 22ng.L⁻¹) were found in all water samples along the continuum. In this same water samples, contamination by both antibiotics and antibiotic-resistant faecal bacteria was mainly related to the land use: structure of E. coli population including occurrence of class 1 integron and the diversity of Enterococcus sp depended on the cow stalling or grazing and the human density.

Our results showed that (i) a simple relationship does not exist between the most detected antibiotics in waters and the most frequently used antibiotics (ii) antibiotic-resistant faecal bacteria are not selected in aquatic environments (iii) bacterial diversity depended on land uses and hydrological conditions.

397A Effects of disease on the composition of sponge-associated bacterial communities

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Reports of diseases affecting marine sponges have increased in recent decades but little information exists on the etiologic agents responsible for these conditions. Many sponges host diverse communities of microorganisms within their tissues, making identification of pathogenic organisms more challenging. Previous investigations of Aplysina Red Band Syndrome (ARBS), which affects Caribbean rope sponges, indicated that the rust-color associated with the leading edges of the lesions is likely due to the presence of filamentous cyanobacteria, that the condition is readily transmissible between sponges via contact, and that the bacterial communities associated with both healthy and ARBS-affected sponge tissues vary over space and time. Terminal restriction fragment length polymorphism and clone library sequence analyses were performed on the bacterial communities of both healthy and ARBS-affected samples, but the bacterial assemblage was highly variable and the etiologic agent (or agents, as this may be a polymicrobial disease) of ARBS could not be readily determined. To provide additional resolution, high throughput sequencing was utilized to offer more insight into how the bacterial community changes when the host sponge is infected with ARBS. Five healthy sponge samples and five ARBS-affected Aplysina cauliformis samples were PCR amplified using universal primers 785F and 1071R and subjected to 454 sequencing. Resulting sequences were assembled into contigs with at least 10 sequences per contig and compared against the GenBank database using BLASTn searches. Preliminary analyses indicated that both healthy and ARBS-affected sponges support a diverse community of microorganisms. Many of the numerically dominant
sequences were closely related to sequences of uncultivated bacteria retrieved from other sponge hosts, providing further support for the existence of specific bacterial genera found predominantly within sponges. Members of the Chloroflexi were well represented in both healthy and ARBS-affected samples while a Pseudomonas sp. appeared in far greater number in the diseased samples than in the healthy samples. Continued examination of the resulting data will help to identify potential etiologic agents of ARBS and develop cultivation media for their recovery.

398A Host-microbes interplay involved in the resolution of intestinal inflammation
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At the present, as many as 4 million people worldwide suffer from inflammatory bowel disease (IBD), which is a chronic disorder of the intestinal tract. Abnormal recognition of commensal gut bacteria has been proposed as one of potent factors in the pathogenesis of IBD. On the other hand, some host-commensals interactions are known to be required for the induction of colitis remission. Thus, we determined on the relationships between host and commensal bacteria during the intestinal inflammation in a DSS (dextran sulfate sodium) induced-colitis model in C57BL/6 mice. After 4 days of DSS administration, we observed the clinical sign of colitis, such as loose stool and bleeding. After that, four different antibiotics (i.e. ciprofloxacin, lincomycin, metronidazole, vancomycin) were administered for a selective change of microflora in mice. Furthermore, four antibiotics combination (ampicillin/metronidazole/neomycin/vancomycin, A/M/N/V) administered for the elimination of intestinal microflora. As a result, a significant loss of body weight was observed in the DSS alone and DSS+A/M/N/V treatment groups. These results indicate that DSS itself could be one of a trigger for colitis, and host-microbes interaction is important for the remission induction. Interestingly, the colitis progression was dramatically suppressed by a lincomycin or vancomycin treatments, suggesting that some microbes play an important role in the remission induction. The colonic microflora in each group were compared for screening candidates of proinflammatory or protective bacteria by a pyrosequencing-based analysis of the V2-V3 16S rRNA gene region. From the results of molecular ecological data, the non-pathogenic Enterobacteriaceae may play an important role for host protection. Interestingly, Lactobacillus species were abundant only in the colon of mice administrated with vancomycin. Next, we focused on how intestinal mucosal immunity is affected by the difference of intestinal microflora, and then characterized interactions between pathogen recognition receptors and commensals with the NFKB-luciferase reporter cell of RAW264 cell. Interestingly, we found a high immune-stimulatory activity of colonic microflora in mice under remission state. From the result of colonic microflora data, these results might be due to the increase of the high-potency endotoxin by overgrowth of non-pathogenic Enterobacteriaceae. The hypothesis of mechanism for remission induction has been formulated based on colonic ecological data as below. In short, the inflammation response is initiated by a translocation of luminal antigen like microbes, and host-mediated inflammation alters intestinal environments from anaerobic to aerobic conditions. Then, this environmental change is thought to allow the overgrowth of aerobic bacteria like E. coli. By a rapid increase of high-potency endotoxin, mucosal innate immune cells (e.g. macrophage) enter into a transient endotoxin tolerance state, and impair the production pro-inflammatory cytokines. After that, host enters into the remission state. However, at this time, we have no clear evidence about the induction of endotoxin tolerance. To clarify the relationship between a higher innate immune-stimulatory activity and disease state, we need to carry out further investigation.

399A A Host-Specific Microbiota is Required for Gut Immune Maturation
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Bacteria can interact with the host's immune system and influence the development of health and disease. Germ-free animals that are not colonized with a microbiota have a defective immune system. Here we examined whether the mere presence of bacteria or a host-specific microbiota is required for immune maturation. We colonized germ-free (GF) mice at birth with a mouse gut microbiota (MMb) or a human gut microbiota (HMB). We studied the evolution of gut microbial composition and intestinal immune maturation over several mouse generations by pyrosequencing of 16S rDNA genes and examining immune cells of the small intestine. MMb- and HMB-mice had a similar total bacterial abundance, as determined by quantitative PCR, and aerobic and anaerobic culturing of bacteria. Both, MMb- and HMB-mice displayed similar relative abundances of the major intestinal bacterial phyla.
Bacteroidetes, Firmicutes and Proteobacteria. MMB- and HMB-mice shared most taxa at the genus level within the major phyla; however, the relative abundance of these taxa differed. Moreover, we found striking differences at the OTU level between MMB- and HMB-mice, in particular among Firmicutes, over the first three mouse generations. Segmented filamentous bacteria (SFB) were detected in MMB-mice, but only partly responsible for immune maturation. Innate and adaptive immune maturation in HMB-mice resembled that of GF mice, and HMB-mice were more susceptible than MMB mice to gastrointestinal infection. Altogether, our observations suggest that mammalian hosts have coevolved with specific consortia of bacterial species, which are critical for development of a healthy immune system.

400A Global patterns of the standing and active human gut microbiome in health and inflammatory bowel diseases
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The knowledge of species composition and functional aspects of the human microbiome is steadily increasing, but little is known regarding the variation in these factors on a global scale. The complex microbial communities inhabiting our body perform a wide array of functions influencing the health of the human host and are also known to play an important role in the development of chronic diseases such as Crohn disease and ulcerative colitis. As these diseases are rising in frequency globally, general patterns may be of clinical relevance, but the stratification of these patterns according to different human populations is of extreme importance. In this study we have analyzed a set of 90 mucosal biopsies sampled from individuals of German, Lithuanian and Indian origin using 454 pyrosequencing of the bacterial 16S rRNA gene. Within each geographic sample of 30 individuals, 10 individuals each of healthy controls, Crohn disease and ulcerative colitis patients were included. From each individual sample DNA and RNA were analyzed as proxies for standing and active microbial community structure, respectively. Using ecological analyses we found strong geographic as well as global disease patterns in the abundance of major phyla and patterns of diversity, which differ when investigated in the standing or active bacterial community. Geography appears to dominate beta diversity, with European samples (that is Germany, Lithuania) appearing much closer to each other, and this is more pronounced in the active microbial community. However, when these geographic influences are accounted for, a universal pattern of disease status becomes apparent, characterized by species clusters restricted to each of the three conditions. We also evaluated the presence of enterotypes in our data and found differences with respect to the standing and active community members. These analyses reveal the importance of the interaction of geography and disease in the interpretation of disease-associated changes in microbial communities.

401A Investigating the impact of biodiversity on clinically-relevant bacterial communities: is more truly merrier?
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The health and economic impacts of human respiratory tract infections are profound. Whilst this represents many different infections, the chronic airways infection that occurs typically in individuals with cystic fibrosis by adulthood has been the focus of much attention. These infections are responsible for much of the high level of mortality associated with this condition. Studies of the microbiota present in the cystic fibrosis airways are documenting an ever increasing number of bacterial species. Little is understood about how these species interact and their importance in relation to the progression of cystic fibrosis associated lung disease. Knowledge obtained through ecological study may be of interest here. These studies have shown that complex relationships exist between biodiversity and community impact. Through these studies, whether in macroecological or microecological scenarios, the community impact as an entity is greater than the sum of the individual species present. Other phenomena are therefore at work rather than simple additive effects. These
phenomena, collectively termed interactions, form the focus of this study. Here, we tested two hypotheses.

1. The total respiration of the community increases additively with increasing biodiversity,

2. Species assemblages have greater activity in more viscous environments.

To test these hypotheses, bacterial species were first cultured aerobically on Muller Hinton and Blood agar from sputa collected from three individuals with cystic fibrosis. From a total of 200 ribotyped isolates, a single isolate of twelve dominant bacterial species was selected. These species were partitioned randomly into six species richness levels in a series of microcosms varying in nutrient content and viscosity taken to reflect the respiratory tract. Generation of carbon dioxide, taken as an indicator of total activity in a microcosm, was measured using the MicroResp™ system. In total, 196 microcosms were analysed with independent replication. Data were analysed using a General Linear Model. Results of this study have shown that as species richness increased, then the % CO₂ produced also increased. The regression model accounted for a modest portion of the variance in the viscous environment (R² ~ 0.4) and less in the non-viscous environment (R² ~ 0.3). Individual species effects and interactions were tested using a variety of ecological models. Results show additive effects with diminishing returns, distinct species effects, a strong role for interactions, and a profound influence of founder effects. In a chronic infection context then, an effective therapeutic has to take account of, or may even exploit, the state of the assemblage.

401B Effect of black tea and fractions on human colonic microbiota measured by in vitro models
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Tea is a popular beverage globally and second only to water in terms of volume consumed. Various studies have implicated black tea in having positive effects on the digestive system in terms of reducing incidence and severity of diarrhea. Previous in vitro studies support anti-microbial properties of black tea components whereby certain potentially pathogenic bacteria were inhibited. In the present study we investigated the impact of 3 different doses of black tea (2, 4 and 6 cups per day) on the human gut microbiota in a validated 3-stage in vitro colonic model that simulates the ascending, transcending and descending colon. A digestion step simulating the stomach and small intestine with dialysis was performed on the black tea in advance. Quantitative PCR was used to measure gut microbial groups and short chain fatty acids were measured by NMR. Surprisingly, the black tea demonstrated a prebiotic effect, that is enhanced bifidobacteria relative to other gut microbial groups, at 6 cups per day. The effect was reproducible. An increase in the short-chain fatty acid acetate occurred together with the bifidogenic effect. Lower doses of black tea, that is 4 and 2 cups, did not appear to give such an effect. Further gut microbial analysis following the 6 cups of black tea using pyrosequencing of the 16S rRNA gene showed a wider impact of the black tea extract, such as increased Ruminococcus, Bacteroides and Actinobacteria, and decreased Prevotella and Faecalibacterium. To gain more insight in the bifidogenic specific effect, fractions of black tea were isolated, namely black tea polysaccharides as well as a black tea fraction enriched for thearubigins and flavonol glycosides. All were tested in batch colon models, along with controls such as no substrate, inulin and whole black tea. The tea polysaccharides increased all the gut microbial groups with no specific selectivity, the black tea as expected gave a bifidogenic effect although not as strong as that of inulin, but the fraction extract enriched for thearubigins and flavonol glycosides selectively gave an enhanced prebiotic effect relative to black tea. It is unusual for a mixture that contains no overt non-digestible polysaccharides to give a prebiotic effect and this is the subject of further studies.

402A Does microbial community structure mediate pathogen colonization in nurses' hands?
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Healthcare workers, by necessity, wash their hands very frequently and often wear gloves. The irritation caused by frequent handwashing and/or wearing of gloves promotes changes on the hands'
microbiota. Irritation can modify the diversity of microorganisms present and enhance colonization by nosocomial pathogens, partly because washing damaged skin is less effective at reducing the amount of bacteria present. Consequently, the proportion of organisms shed is oftentimes higher, increasing the potential for transmission. We investigated the variability of hand microbiota between and within 34 surgical intensive care unit healthcare workers at three time points, from glove-juice samples and swab samples collected between July 5-18, 2011. Glove-juice samples were cultured for viable hand microbiota quantitation, showing no growth on MacConkey's agar and a range of 1x10² to 5x10⁴ CFU/ml on trypticase soy blood agar. To examine the variability between and within individual healthcare worker through time, we sequenced the V6 hypervariable region of the 16S rRNA gene using Ion Torrent technology with a 316 chip on the Ion Personal Genome Machine™ (PGM™) Sequencer. We also screened samples for the presence of known nosocomial pathogens using real-time qPCR. Bioinformatics analysis is ongoing. We also investigated whether the microbial community structures found on the healthcare workers’ hands was related to environmental variables. Hand hygiene practice, levels of patient contact, self-assessed hand health and socio-demographics were assessed using a self-administered questionnaire at baseline. We also visually rated hand health using a validated assessment tool (VSS). The healthcare workers (26 female and 8 male), aged 20-59, were mostly in excellent or good health. Most, on a typical 12-hour work shift, cleaned their hands 6-20 times with soap and water, and >40 times with alcohol rub. Most also donned a pair of gloves > 40 times per shift. While most reported directly caring for an average of 1-2 patients during a typical work shift, varying levels of invasive procedures, from turning patients and handling soiled linen, to caring for IV/urinary catheter, drain, and/or endotracheal tube sites, were performed on a routine basis. We will integrate metadata results with sequencing analysis to address whether microbial community structure mediate pathogen colonization in nurses’ hands.

403A  Microbial drivers in the development of black band disease in coral
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Black band disease (BBD) is a polymicrobial infection affecting corals worldwide and causing localised mass mortality particularly in summer warm temperatures. The microbial mechanisms contributing to the development of BBD are poorly understood.

Characteristic cyanobacteria-dominated lesions, termed cyanobacterial patches (CP), were found to precede the onset of BBD in ~20% of cases on a reef within the central inshore Great Barrier Reef. Slower progression rates of CP than of BBD indicate that the virulence of lesions intensifies as BBD develops from CP. Concurrently, microbial communities within lesions exhibit transitional changes, including a shift in the dominant cyanobacteria and increase of the abundance of sulphate reducing bacteria (SRB). Bacterial and archaeal communities within CP and BBD and associated microenvironmental parameters were investigated to understand the drivers of microbial dynamics in the development of BBD.

Chemical profiling of the lesions using microsensors illustrated that CP mats are oxygenic and devoid of sulphide in general, whereas BBD mats are anoxic and highly sulphidic in darkness and at low light levels, confirming these microenvironmental factors are key in the development of BBD. Quantitative PCR targeting the dissimilatory sulphite reductase gene (dsrA) showed that the abundance of dsrA gene copies increased in the transition from CP to BBD. Clone libraries of archaeal 16S ribosomal RNA genes also showed a shift from diverse aerobic marine populations in CP to ones dominated by novel archaea in BBD, peripherally related to strictly anaerobic methanogens and potentially syntrophic to SRB. Comparative metagenomic approaches to CP and BBD further identified other key players in BBD pathogenicity. These results highlight the importance of the whole microbial communities in the pathogenesis of BBD which include sulphur-cycle-related microorganisms.
404A Biocontrol effect of bacterial antagonists towards Rhizoctonia solani on lettuce and interaction with the indigenous rhizosphere communities affected by soil types?

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The soil-borne pathogen Rhizoctonia solani causes bottom rot on lettuce with the result of crop losses and reduction in crop quality. In general strategies to control Rhizoctonia diseases are limited. The use of fungicides is critical under consideration of pesticide residues in the final food product. Therefore, the development of alternative control strategies is urgently necessary. A promising approach is the use of efficient antagonists able to suppress the disease. In previous studies the interesting antagonists Pseudomonas jessenii RU47 and Serratia plymuthica 3Re4-18 with efficient disease suppression effects against R. solani in lettuce were selected. In general, the efficiency of biocontrol agents varied under field conditions and the reason for this variability is largely unknown. Therefore, a better understanding of the complex interaction between antagonists and the plant rhizosphere microbial community under consideration of the soil type is required for a successful exploitation of this antagonistic potential. Field experiment has been set up with a unique plot system comparing the impact of three different soil types on the lettuce growth, bottom rot disease severity and especially on the complex microbial interactions. The influence of the soil type can analyze independently from other factors such as climate and cropping history. First results showed that the pathogen has a strong effect on the plant growth in all soil types tested. Both antagonists colonized effectively and in a comparable density the rhizosphere of lettuce and decreased significantly the disease severity of bottom rot independent from the soil type. In contrast, denaturing gradient gel electrophoresis (DGGE) revealed highly significant differences in bacterial and fungal community pattern between the soil types. But the indigenous microbial community was not affected by the antagonist application. Hence, a negative ecological effect is excluded by antagonist application. However, the biological mechanism remains unknown. In conclusion the strains are promising biocontrol agents and can include in disease management.

405A HuMiX: a microfluidics-based in vitro co-culture device for investigating human-microbial molecular interactions

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Mixed microbial communities play pivotal roles in governing human health and disease. While omic analyses have highlighted differences in the human microbiome between both states, causal links are difficult to ascertain because of a distinct lack of in vitro human-microbial co-culture systems in which emergent hypotheses can be tested.

We have recently developed a modular microfluidics-based device (HuMiX) that allows the partitioned but proximal (< 200 μm) cultivation of human epithelial cell lines and sampled human microbial communities while at the same time allowing molecular interactions between both contingents across semi-permeable membranes. The device dimensions ensure laminar flow profiles, allowing us to create two micro-niches (anaerobic for microbes and aerobic for human cells) in the respective culture chambers.

The HuMiX device includes 3 separate chambers of which the middle chamber is inoculated with human epithelial cell lines [Caco-2 and HT29-MTX (90:10) totaling 10^7 cells in 200 μL of media]. The bottom chamber is a dedicated perfusion chamber separated by a micro-porous membrane, which facilitates diffusion-based perfusion to the basal aperture of the human epithelial cells. The human contingents are perfused with DMEM medium supplemented with Fetal Calf Serum (10-20%) at 50 μL/hr flow rate using a programmable syringe pump. The microbial contingents are introduced in the top chamber after the Caco-2 and HT29-MTX cells form a monolayer and undergo an enterocytic differentiation mimicking the intestinal epithelial barrier (approximately 2 weeks). Faecal inoculates are extracted by washing samples from healthy human donors with phosphate buffered saline solution. The microbial chamber is coated with porcine mucin and perfused with gastrointestinal microbial medium to facilitate growth of a diverse and representative microbial community in the device. The
The semi-permeable membrane facilitates efficient crosstalk (mediated by extracellular proteins and peptides, nucleic acids, and metabolites) between the two contingents and the co-cultures are continually evaluated via confocal microscopy and metabolomics (on spent medium). Once both the contingents reach a steady-state, the devices are used for targeted perturbations. Following such experiments, the devices are snap frozen and the respective cell contingents undergo comprehensive bio-molecular extractions for metabolites, DNA, RNA and proteins. Consequently, the modular device architecture allows independent probing of the individual cell contingents using the latest high-resolution omic analyses.

In conclusion, the HuMIX device enables proximal co-culture of faecal microbial organisms in close proximity with human epithelial cells while at the same time maintaining two microniches separated across a nanoporous membrane. In the near future, we aim to employ the device and the related biomolecular extraction protocol to leverage our understanding of fundamental molecular processes related to syntrophy and antagonism between human and microbial cells as well as the resulting effect of these processes on human health and disease states. In particular, we aim to uncover the potential role of microbial dysbiosis in pathogenesis of different diseases.

406A In vitro actinomycetes biofilm development and biofilm inhibition by polyene antibiotic, nystatin on IUD copper surfaces
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The presence of intrauterine contraceptive devices (IUDs) gives a solid surface for attachment and an ideal niche for the biofilm to form and flourish. Pelvic actinomycosis is often associated with the use of IUDs. To treat pelvic actinomycosis which develops in connection with IUD wearing requires the immediate removal of IUD. Therefore this article presents in vitro evidence to support the use of novel antibiotics in the treatment of actinomycetes biofilm.

The clinical actinomycetes isolates from the endocervical swabs of IUD wearers was assessed for the biofilm forming ability. The effect of nystatin on the disruption of biofilm was assessed by crystal violet assay.

A total of 21 actinomycetes were isolated from the endocervical swabs of the IUD wearers. Of these 21 isolates, 3 isolates showed high mature biofilm forming ability. An in vitro biofilm model with three isolates A4, C15 and C17 was subjected for the treatment with nystatin. Inhibition of biofilm by nystatin was found to be concentration dependent with MBIC50 values in the range of 0.08 -0.16 mg/ml. Further at a concentration of 0.16mg/ml, nystatin inhibited the twitching motility of the isolates evidencing the possible mechanism of biofilm inhibition.

The results of the present study envisage the protective effect of nystatin on the disruption of biofilm. Hence nystatin can be recommended for the treatment of actinomycetes infection or nystatin coated IUDs can be used to completely eradicate pelvic actinomycosis.

405B QTL analysis of the skin microbiota and gene-microbe interactions in susceptibility to experimental epidermolysis bullosa acquisita (EBA)
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The skin is in constant contact with the environment and serves a critical barrier function, yet provides a range of niches to inhabiting microbial communities. A multitude of interactions between the skin microbiota, host and environment contribute to community structure and its potential contribution to
changes in health status. Inflammatory disorders of the skin harbor clear immunogenetic components, but whether these associations may be mediated by alterations in microbial community structure is unknown. Epidermolysis bullosa acquisita (EBA) is a chronic skin blistering disease of autoimmune origin characterized by antibodies to type VII collagen (COL7). In this study, we made use of the fourth generation of an advanced, 4-way, autoimmune-prone intercross line (G4 AIL) mapping population, for which a QTL analysis of EBA already exists, to perform a quantitative trait locus (QTL) analysis of the skin microbiota. Microbial “phenotyping” of mouse ears was carried out using barcoded 454 pyrosequencing of the bacterial 16S rRNA gene in 261 individuals. After defining a “core measurable microbiota” of 131 species level OTUs, we identified a set of 235 overlapping QTLs for 97 of these OTUs. Furthermore, we identified significant overlap between bacterial and EBA susceptibility QTLs, and a covariate analysis between bacterial abundances and EBA scores displayed evidence of host gene-microbe interactions contributing to this susceptibility.

407A  Changes in oral microbiota profiles associated with caries : a pilot study
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Dental caries is a polymicrobial infection: the progression from healthy teeth to dental caries is associated with shift in the bacterial community structure. We previously demonstrated using denaturing gradient gel electrophoresis that bacterial communities from healthy children who proceeded to caries are different from those that remain healthy. To confirm these findings, we used next generation sequencing with an Illumina paired end protocol. Dental and tongue samples were obtained from 5 children who were caries free at both visits and 11 children who were caries free at the first visit and developed dental decay by the second visit. All children were enrolled in an oral health disparities study at the Center for Oral Health Research in Appalachia. We sequenced a 100 bp fragment amplified from the V6 region of 16 S ribosomal gene from teeth and tongue samples. All samples were amplified and sequenced in duplicate to ascertain error introduced during PCR amplification and Illumina sequencing. A total of 5,921,128 reads were generated and ranged from 4515-154771 reads per sample. Average number of OTUs defined at 97% similarity ranged from 335 OTU on teeth (range: 223 - 406) and 340 OTU on tongue (range:198 - 415) respectively. At 100% identity, an average of 625 and 649 OTUs were defined for the teeth (range: 313-790) and tongue (range: 304-825) respectively. Shannon and Simpson indices, evenness and expected number of species present were calculated using Vegan package in R. Bacterial communities present on teeth and tongue were correlated (Spearman rho = -0.49, p value= 0.055). Shannon diversity indices were correlated within replicates of same samples (Spearman rho = -0.24, p value 0.09 ) but membership and Simpson diversity indices were not correlated (Spearman rho and p- value = -0.13, 0.38 and - 0.17, 0.23 respectively). One explanation for the differences in within-sample replicates is attributable to sequencing artifacts that increase the number of rare OTU’s detected. The differences in the sensitiveness of Shannon and Simpson indices to rare OTUs could potentially account for why the Shannon index were better correlated within sample replicates. Differences in diversity indices could also be due to PCR introduced errors in sample replicate sequences; work is ongoing to explore the underlying cause for within sample variability. Community analysis using non-metric multidimensional scaling are planned to identify specific OTUs associated with caries associated changes in bacterial communities.

408A  Bacterial community succession on a hydroxyapatite disk in oral cavity in caries-active and caries-free subjects
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Dental caries is one of the most common infectious diseases, whereas several studies have shown that potentially pathogenic microbial communities rather than a single pathogen would be involved with its development. Thus, the interaction between microbes within microbial communities must be critical for virulence of microbial community. However, succession of cariogenic plaque microbial communities remains uncharacterized.
In this study, using a retrievable hydroxyapatite disk model, plaque samples accumulated on the disk after 1, 2, 3, 4, 5 and 7 days were obtained from 9 caries-free and 10 caries-active (with ≥9 teeth with caries experience) subjects (23 ± 6 years, 6 females and 13 males). The bacterial community structure was investigated by barcode pyrosequencing analysis of 16S rRNA gene.

In both caries-active and -free groups, facultative bacteria, including *Streptococcus* appears to predominate during the early stages and anaerobes such as *Prevotella*, *Veillonella* and *Porphyromonas* increased dominance in the late stage. On the other hand, microbial diversity in Day 1 of caries-free group was significantly higher than that of caries-active group, and relative abundances of *Neisseria* and *Gemella* in caries-free subjects were greater than caries-active subjects in the early stages. In addition, *Granulicatella*, *Veillonella* and *Actinomyces* were significantly less predominant in caries-free subjects in the late stages compared with caries-active subjects.

These results revealed characteristic microbial succession pattern of dental plaque in caries-free subjects. It might lead to their low susceptibility to dental caries.

**041A  Genome-wide mapping of transcription factor binding in Pseudomonas aeruginosa**
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The opportunistic pathogen *Pseudomonas aeruginosa* is a Gram-negative bacterium found in diverse environments such as soil, moist environments and plants. *P. aeruginosa* is the most common cause of pulmonary infections in patients suffering from the genetic disease cystic fibrosis. The ability of the bacteria to adapt to changing ecological conditions is facilitated through a very large repertoire of regulatory genes responding to environmental cues. Moreover a number of RNA polymerase binding sigma factors are important regulators of life-style changes made necessary in cases of more profound shifts between different environments. The transcription sigma factor RpoN (σ^54) is suggested to play a role in survival to environmental stresses during the transition from one environment to another. From transcriptomic studies of RpoN it is known that this sigma factor is a key regulator of virulence factor gene expression and it also regulates many genes involved in the transport and metabolism of diverse carbon and nitrogen sources. However, whether these regulatory effects are direct or indirect is not clear for most of the affected genes. Here we present the first genome-wide mapping of RpoN-DNA binding interactions in *P. aeruginosa* using chromatin-immunoprecipitation and sequencing (ChIP-seq). The information gained from this study can be used to specify the role of RpoN in the infection process of the bacteria in the cystic fibrosis airways. This type of analysis will furthermore be a step forward in the characterization of the complex regulatory network of this bacterium.

**408B  Impact of rye and wheat used as mulch in a cover crop production system on the Pseudomonas syringae diversity**
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Cover crops are planted to manage soil fertility, soil quality, water, weeds, disease and biodiversity in agrosystems. As part of a project aiming to explore factors that contribute to reduce the disease severity caused by *Pseudomonas syringae* pathogenic to squash, we hypothesized that cover crops could sustain and promote bacteria that can compete with the pathogen. We initially carried experiments on the use of cover crop with the aim to reduce disease in Spaghetti squash. Winter rye and wheat were used as cover crops and were seeded during fall for the next growing season. In spring, cereals were grown until they reached the milky stage and were terminated using a roller crimper, some treatments included a glyphosate spray before termination. The squash were then transplanted into the mulch formed by cover crops. The control treatment was a traditional cropping system (bare soil). The incidence and severity of symptoms caused by a pathovar of *P. syringae* was drastically reduced by cover crop under high disease pressure compared to the control (2008, 2011). In 2011, squash seeds were soaked in a 10^5 CFU/ml suspension of a rifampicin resistant *P. syringae* to assure disease presence. Following these results, we scouted for *P. syringae* isolates on the premise that this specie is adapted to compete with the squash pathogenic *P. syringae* pathovar. The first survey was conducted at the end of 2011 season. Based on a preliminary identification using LOPAT profile, 54 isolates of *P. syringae* were recovered from squash leaves and 24 from rye and
wheat straw. All isolates recovered from straw were not resistant to rifampicin, indicating that they were not the strain used to contaminate the seeds. On the other hand, 80% of the strains isolated from squash were resistant to rifampicin and they were recovered in all treatments while the non-resistant strains were isolated only from squash grown in rye and wheat. Of this collection, 26 strains were compared using BIOLOG Gen III microplates and the hierarchical clustering distinguished three clusters. The first cluster included 14 strains, among them the 13 strains resistant to rifampicin. All of them were isolated from squash and showed pathogenicity on squash using artificial inoculation. All isolates of clusters two and three were not-resistant to rifampicin. The four strains of cluster two were isolated from squash grown in rye and isolates from cluster three were mostly isolated from mulch straw. These results indicate that there is diversity among the P. syringae isolates recovered in the rye and wheat treatments, which is not observed in the isolates recovered from the control treatment thus confirming our hypothesis that cover crops can favor antagonist strains. Characterization based on the CTS gene is currently under investigation for isolates collected in 2011. Further strains will be collected over the next years to draw further conclusions.

409A  Diversity of nontuberculous mycobacteria in unchlorinated drinking water
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Certain members of the nontuberculous mycobacteria (NTM) are opportunistic pathogens that cause pulmonary infections in immunocompromised persons. Several studies showed that the opportunistic pathogenic species Mycobacterium avium, M. kansasii and M. xenopi can be present in drinking water. However, information about NTM population composition in drinking water is still lacking. Therefore, the objective of our study was to determine NTM communities in unchlorinated drinking water from the distribution system of six different treatment plants in the Netherlands.

DNA was isolated from treated water and drinking water samples taken from the distribution system. Subsequently, the hsp65 gene of mycobacteria was amplified using previously published specific primers for Mycobacterium and multiple amplified hsp65 genes were sequenced using 454 sequencing.

In total, 12,310 hsp65 gene sequences were obtained from all 24 sites. However, only 3,369 sequences belonged to mycobacterial species. Thus, the primers were not specific to the hsp65 gene of Mycobacterium species, which was unexpected since these primers have been used by others to determine NTM in drinking water and other environments.

Analysis of the 3,369 mycobacterial hsp65 gene sequences showed that the diversity in unchlorinated drinking water was high and up to 23 different Mycobacterium species could be observed in a single drinking water sample. Most NTM hsp65 gene sequences (96.8%) were related to not-yet cultivated mycobacterial species, and their role in public health is probably low, since these species have not been cultured from patients. The other sequences (3.2%) showed more than 97% similarity to sequences from M. gordonae, M. lentiflavum and M. asiaticum, species that have been identified in immunocompromised patients, and M. salmoniphilum, which is pathogenic for fish.

The diversity was higher in drinking water from the distribution system and in the summer than in treated water or in the winter. The similarity between NTM communities in drinking water from three different locations in one distribution system taken in winter and summer was 7.8 to 75.8%. The similarity of these NMT populations at the three locations in the distribution system was 20.3 to 53.5% and 21.5 to 49.8% in the winter and summer, respectively. In addition, the NTM communities observed at the same location in the distribution system showed 30 to 67% similarity between winter and summer samples. Comparable observations were made for most of the other distribution systems and between these distribution systems. The high diversity and low similarity suggest that numerous factors influence the establishment of an NTM community in drinking water distribution systems. Most likely, biodegradable organic matter composition and concentration, pipe material and length, residence time, sediment, biofilm, temperature and season play a role in the establishment of an NTM population in drinking water distribution system.

Our study revealed that (i) mycobacterial populations in unchlorinated drinking water samples are diverse and differ considerably between distribution systems, locations within a distribution system and
season, and (ii) only a small number of the observed NTM are related to species that are pathogenic to immunocompromised humans.

410A  Rodent species as reservoirs of Borrelia human pathogens in Dutch natural areas
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Lyme borreliosis is the most prevalent emerging zoonosis in temperate regions of the northern hemisphere. The disease is caused by the bacterium Borrelia burgdorferi sensu lato that is transmitted via Ixodes ricinus ticks to humans. These ticks also can carry numerous other pathogens apart from Borrelia species. Rodents play key roles in the circulation of human pathogenic Borrelia species in natural areas. The purpose of this study was to find an eventual relationship between infection prevalence in captured rodents, in ticks feeding on these rodents and in ticks questing in the vegetation. Therefore, rodents were captured in three natural areas in The Netherlands, that is ‘Amsterdam water supply dunes’, ‘De Hoge Veluwe’ and ‘De Kwade Hoek’, and Borrelia species-infection prevalence was investigated in these rodents and in feeding and questing ticks.Questing ticks were found in all three areas, although numbers of the different growth stages differed per area. Borrelia species prevalence in questing ticks varied between zero and 20% and these values did not correspond with the ones recorded over time spans of several months in the same areas (between 3.7 and 39.4%). Borrelia afzelii was the most prevalent among the Borrelia species found in ticks. Apodemus sylvaticus (wood mouse) and Myodes glareolus (bank vole) were the dominant rodents captured, although M. glareolus was absent in one area. Of all captured rodents, 96% was infested with ticks, and larvae were most frequently found feeding on both rodent species. Infection prevalence in ear biopsies of both rodent species varied between zero and 33.3%, and percentages corresponded with the ones found in questing ticks sampled over longer time spans. Infection prevalence in feeding larvae varied between zero and 29.4%. Infection prevalence in larvae feeding on A. sylvaticus from the three areas corresponded with infection prevalence found in ear biopsies of the same rodent species. An interesting finding was that a significantly higher number of nymphs was present on Borrelia species-infected A. sylvaticus individuals (1 per individual) than on non-infected ones (0.5 per individual). This would indicate the existence of a mechanism that favors Borrelia species-infected A. sylvaticus to become infested with higher tick numbers than non-infected ones. Infection rates in questing ticks sampled over longer time spans and in A. sylvaticus individuals was always highest in one particular area, ‘De Kwade Hoek’. Elevated infection prevalence in questing nymphs and rodents, such as present in the ‘De Kwade Hoek’ area, can be explained by the fact that the landscape is relatively open; there is no tree cover and transitions between vegetation types are sharp. Such heterogeneous habitats may facilitate circulation of vector-borne diseases more efficiently. It was therefore concluded that Borrelia species transmission between ticks and rodents is more efficient in that area and that infection prevalence in feeding larvae represent the infection prevalence of the upcoming generation of nymphs; the stage that is most responsible for the transmission of pathogenic Borrelia species to humans.

411A  A gene-targeted approach to investigate the intestinal butyrate producing bacterial community
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Gene-targeted analysis enables the direct investigation of microbial functions and became common practice in microbial ecology. In this study we present an approach for 454 pyrotag sequencing and quantitative PCR (qPCR) targeting the final genes in the two primary bacterial butyrate synthesis pathways, butyryl-CoA:acetate CoA-transferase (but) and butyrate kinase (buk). Furthermore, we established a gene-targeted metagenome assembly tool for those genes where a De Bruijn Graph representation and a protein profile Hidden Markov Model (HMM) are combined. The initial establishment of butyrate producing communities in four ulcerative colitis patients who underwent colectomy with ileal pouch anal anastomosis was monitored over two months and compared to three control samples from healthy colons. All patients established an abundant butyrate producing community (approximately 5 to 20% of the total community) in the pouch over time, but community...
profiles were distinctive among individuals. Only one patient harbored a community profile similar to the healthy controls with but genes predominating similar to reference genes from Acidaminococcus sp., Eubacterium sp., Faecalibacterium prausnitzii and Roseburia sp. and an almost complete absence of buk genes. Two patients were greatly enriched in buk genes similar to those of Clostridium butyricum and C. perfringens, whereas a fourth patient displayed abundant communities containing both genes. Most butyrate producers identified in previous studies were detected and the results were supported by 16S rRNA gene pyrotag analysis, but only the gene-targeted approach provided the depth necessary to reveal the entire butyrate community profiles.

**412A** Bacterial profiling of White Plague Disease in a comparative coral species framework
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Coral disease heavily impacts reef health and has reduced live coral cover by up to 80% in some places. We are well aware of the high diversity and specificity of coral-associated bacteria. However, there are only few studies that have looked at changes within the bacterial community of corals as a consequence of disease. Furthermore, most studies have focused on microbial profile shifts of a specific coral species that suffers from a specific disease.

Here we aim to apply an integrative approach to the study of White Plague Disease (WPD) by analyzing microbial profiles of different coral species via 16S PhyloChip assays. Healthy and WPD-displaying corals of Pavonasp. and Porites lutea from the same reef in the Gulf of Thailand were analyzed in order to determine whether core microbiomes in coral health and disease exist.

Our data indicates that a common core microbiome of coral health and disease exists that is evolutionary conserved over coral species boundaries. While corals do host a complex bacterial community that is species-specific, corals display shared bacterial community profile changes as a consequence of shifts from healthy to diseased states. This is the first study that analyzes coral disease in a comparative species framework, and a first step towards establishing common core microbiomes in coral health and disease.

**413A** Scenedesmus-Microcystis interaction may provide a novel approach to combat toxic cyanobacterial blooms
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Microcystis sp. is a major player in the global intensification of toxic cyanobacterial blooms endangering the water quality of fresh water bodies. A novel green alga identified as Scenedesmus sp. designated strain HUJI (hereafter S. HUJI) was isolated from Lake Kinneret, Israel, and was found to secrete allelochemicals that lyzed toxic Microcystissp. strains. A new separation procedure was developed and applied in order to concentrate the active compounds from S. HUJI spent medium. In nanogram quantities they interfere with the functionality and perhaps the integrity of the cell membrane, as was indicated by the rapid effect on the variable fluorescence and leakage of phycobilins and ions. The isolated active compounds were shown to act specifically against cyanobacteria and the gram-negative E. coli. They neither affected a gram positive Bacillus sp. nor caused hemolysis of red blood cells or altered growth of other eukaryotic algae such as Chlamydomonas reinhardtii. The newly developed isolation procedure and compounds obtained may provide a new means for combating toxic cyanobacterial blooms and for the identification of novel antibiotics.
### 414A  Genus- and strain-level culture-independent characterization of acute uncomplicated urinary tract infections with comparison to clinical isolate data

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Urinary tract infections (UTI) are one of the most commonly acquired bacterial infections in humans, accounting for over eight million doctor visits in the US alone. Uropathogenic *Escherichia coli* strains are responsible for over 80% of all UTI cases, with the remainder attributable to other microorganisms such as *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus*, *Klebsiella*, and *Enterococcus* species. The standard method for identification of uropathogens in most clinical laboratories is cultivation, primarily using solid growth media under aerobic conditions, coupled with morphological and biochemical tests of typically a single isolate colony. However, these methods are only able to detect culturable microorganisms, and characterization is phenotypic in nature. Here, we explored the genotypic identity of communities present in acute uncomplicated UTIs from 50 individuals using culture-independent amplicon pyrosequencing with biological replication. The study subjects represented both males and females and a range of ages from *Escherichia* was the most abundant genus in the study cohort, however *Escherichia*-associated UTI was less common in older males than in younger individuals and females. We next strain-typed *Escherichia*-dominated UTIs using amplicon pyrosequencing of the fimbrial adhesin gene, *fimH*. There were 9 highly abundant *fimH* types across the 50 UTI samples, and each sample was dominated by a single *fimH* type. Molecular analysis of the corresponding clinical isolates for the UTI samples revealed that in the majority of cases the isolate was representative of the dominant taxon in the community at both the genus and strain level. Notable exceptions mostly comprised dominant anaerobic populations that are not obtained through standard clinical cultivation. These results suggest that for many UTIs, single cultured isolates are diagnostic of the infection.

### 414B  Antibiotic resistance in freshwater: should increasing agricultural antibiotic use concern us?

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A key problem challenging public health official's efforts to stem the spread of antibiotic resistance is the potential increase of antibiotic resistance in the environment. Yet paradoxically, the extent of antibiotic resistance in New Zealand's freshwater environment were unknown. Antibiotics are used extensively in agricultural sectors worldwide, with their subsequent detection in waterways traversing agricultural land widely reported. Despite recent and significant changes to agricultural land-use in New Zealand, as well as the sector's high antibiotic use (over 57% of all antibiotics used in NZ in 2000), the effects of antibiotics in the environment remained uncharacterised. Given antibiotics are designed to cause biological effects, a major concern in relation to antibiotic presence in the environment is the potential promotion of microbial antibiotic resistance. Therefore, the potential risk of antibiotic resistance to the public's health from a river used for drinking water, food gathering and recreation, yet subject to increasingly intensive animal agriculture with its concomitant high reliance on agricultural antibiotics, was investigated for one year. Spatial and temporal dynamics of antibiotic resistance in freshwater biofilms from six sites along New Zealand's fourth longest river (and second longest river unmodified by large dams) were examined nine times throughout the year using PCR to gauge the level of resistance present, as well as potentially tease apart the likely contamination sources of antibiotic resistance. Biofilms were screened in their entirety for ten genes conferring resistance to antibiotics used in humans only (vancomycin) and both humans and agricultural animals (aminoglycosides, β-lactams, macrolides and tetracyclines). Antibiotic resistance was detected at the two most downstream of six sites, which in particular are subject to intense animal agriculture, but this only occurred on two sampling occasions in the late summer/early autumn out of a total of 162 samples and for only three genes. Given the low frequency of resistance genes detected, no correlations could be determined between the detection of resistance and physicochemical parameters of the river. These findings indicated that minimal public health risks exist to antibiotic resistance arising from intensive animal agriculture along the river's catchment. However, owing to the detection of a gene conferring resistance to a human-only use antibiotic at a popular recreational site in late summer, the importance and role of human-based antibiotic resistance contamination sources in this river warrant further investigation.
Endophytic fungi may provide their host plants with an epigenetic mechanism of adaptation to environmental stress or protect the hosts against pathogens and herbivores. Forest trees are invaded by endophytic fungi from the environment, and endophytes seem to activate in their hosts the same or similar defence mechanisms as the pathogens. Yet, we do not know whether the degree of a tree’s resistance to pathogens affects the establishment of endophytic fungi within it. Improvement of resistance through breeding could have environmental trade-off effects, potentially cascading from individuals to trophic levels and communities, and modifying ecosystem services that the endophytes provide.

Elm (Ulmus spp.) trees are severely affected by the Dutch elm disease (DED) pathogen (Ophiostoma novo-ulmi). We hypothesized that due to their stronger defences, elm genotypes with a high resistance to DED host a less rich endophyte community than highly susceptible elms.

To test the hypothesis, we isolated fungi from surface sterilized, symptomless tissues (leaves, bark and wood) of elm trees (U. minor, U. pumila) belonging to genotypes that differ in their resistance against DED. We focused on the cultivable fraction of endophyte community as a step towards our long-term goal of finding easily cultured isolates for biotechnological applications. As a defensive trait of elm genotypes, HPLC-profiles of phenolics were compared in corresponding tissues. Isolates were grouped into morphotypes and selected isolates, linked to particular tree resistance patterns, were identified using molecular tools. Ability of certain endophytes to antagonize O. novo-ulmi was evaluated in vitro and in vivo.

A collection of over 200 fungal isolates was established. Several endophytes produced extracellular metabolites, some of which were able to negatively affect the colony growth pattern of the pathogen in in vitro tests. Other possible modes of antagonism exert by the endophytes were mycoparasitism and outcompeting. Resistant and susceptible elm genotypes were differentiated by their xylem chemistry, but not by leaf or bark chemistry. In xylem, but not in leaves or bark, resistant elm clones exhibited a lower frequency and diversity of fungal endophytes than susceptible U. minor clones.

In conclusion, the high diversity and tissue-specificity of endophytic fungi in elms emphasizes the value of these trees as a habitat for microbes. Certain elm endophytes appear to be highly potent biocontrol agents against DED, acting through different mechanisms (chemical antagonism, growth rate difference or mycoparasitism). Endophytes thus constitute promising material for integrated management of DED. The difference in endophyte diversity and frequency between resistant and susceptible elm clones implies that re-introduction of elms to forest ecosystems with the assistance of breeding for quantitative resistance to DED may lead to non-targeted effects on fungal biodiversity. As endophyte diversity may contribute to various ecosystem benefits from forests in a similar way than rhizospheric diversity, this issue should be addressed in environmental impact analyses of tree breeding efforts.
Pseudomonas aeruginosa. We observe that the Pseudomonas population in each patient and at each time point is comprised of multiple isolates with diverse phenotypes. Heterogeneity was observed for every phenotype measured, even within isolates of the same colony type. Clustering of the phenotype profiles of 161 isolates from one patient revealed that the isolates do not group by colony morphology or mucoidy. These data demonstrate that the airways of CF patients support a diverse and dynamic P. aeruginosa population and we suggest that this diversity is indicative of adaptive radiation in the colonized airways.

Detection system of Rotavirus in water samples

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There are viruses that belong to aquatic systems in the environment, in which they make interactions with other organisms and interfere in the kinetics and population regulations. Viruses are usually found in the aquatic environment in very small amounts and their detection is difficult and complicated. For their detection it is necessary a previous step of concentration, what makes necessary to develop a capture system and viral detection for monitoring water samples. Therefore the importance of monitoring the presence of viruses in water, integrating the optimization of a filtration system for the detection of virus. For this reason the aim of this work was to establish a viral detection in water samples.

Samples of one liter of water artificially contaminated with rotavirus at different concentrations were filtered with the Virocap VCM-47®, this filter is a positive charge membrane, of low cost used for viral studies. For the development of the capture system, the absorption-elution method (Viradel) was selected for the concentration virus because it was easier to work than with other techniques currently used. Samples were eluted with beef extract, 1% Tween80-0.5M glycine pH9 and then a secondary concentration by centrifugation, where viral RNA was extracted by the method of Trizol® for the molecular detection of Rotavirus by RT-PCR, the primers CON2/CON3 were used to amplify the fragment of 887bp encoding the VP4 protein of rotavirus. In conclusion, we designed an alternative system of filtration through Virocap VCM-47® membranes eluted with beef extract 1%-glycine 0.5M-tween80 pH9, with a secondary concentration which is capable of detecting Rotavirus in water samples artificially contaminated with them and proved using RT-PCR.