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The Biology of Vibrios: Genomes to Biology

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Abstract Handbook

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Oral Presentations

Session 1 – Ecology of *Vibrios* Part I

Vibrio cholerae in food vacuoles expelled by protozoa are protected from stresses and more infectious in vivo than free-living cells

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Vibrio cholerae interacts with a wide variety of organisms, including heterotrophic protists (protozoa). Several species of these bacterial predators have been reported to release live, undigested bacteria in expelled food vacuoles (EFVs) when feeding on certain pathogens. While the production of EFVs has been reported, their biological role as a vector for the transmission of pathogens remains unknown. Using co-incubation assays, we report that Tetrahymena pyriformis releases large numbers of EFVs when feeding on *V. cholerae*. The EFVs are stable, the bacterial cells within are protected from multiple stresses (low pH, antimicrobials and starvation) and vast numbers quickly escape when incubated at 37°C or in the presence of nutrients. We show that OmpU, a major outer membrane protein positively regulated by ToxR, plays a significant role in the production of EFVs. Importantly, cells released from EFVs have growth and colonisation advantages over planktonic cells both in vitro and in vivo and are highly infectious (as shown in the infant mouse model of infection). Our results suggest that EFVs facilitate *V. cholerae* survival in environment and in the gastric environment, enhancing infectious potential and may significantly contribute to the dissemination of epidemic *V. cholerae* strains. These results establish a new understanding of the mechanisms of persistence and the modes of transmission of *V. cholerae* and may further apply to other opportunistic pathogens that have been shown to be released by protists in EFVs. Results presented here will improve the identification and tracking of pathogens in the environment.

V. vulnificus Type VI Secretion System (T6SS) interactions in an oyster in vivo model and their impact on Vibrio ecology

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V. vulnificus is a significant human pathogen that causes primary septicaemia or wound infections in susceptible individuals. A primary route of infection is the consumption of contaminated seafood, in particular oysters. Two type VI secretion systems, the T6SS1 and T6SS2, have been identified in *V. vulnificus*. This study aimed to characterise the impact of the T6SS on *V. vulnificus* ecology. We have demonstrated that 'attacker' *V. vulnificus* strains are able to target and kill 'prey' strains under a range of temperate conditions using both T6SS1 and T6SS2. To elucidate the impact of the T6SS *on V. vulnificus* ecology we sought to demonstrate that these results are representative of in vivo interactions by developing an oyster model. Oysters are filter feeders and filter and ingest particles in a size-dependent manner, this typically means planktonic bacterial cultures are not retained as effectively as

necessary. We therefore developed a method of incorporating bacterial cultures into larger, phytoplankton-based aggregates termed 'synthetic marine snow', enabling consistent uptake and retention by oysters. To assess in vivo competition, oysters were exposed to synthetic marine snow containing both attacker and prey *V. vulnificus*. Post incubation, oysters were processed and attacker and prey strains enumerated. Killing of the prey *V. vulnificus* strain was observed by the wild-type attacking strain and the T6SS deletion mutants, indicating active in vivo antibacterial activity of both T6SS1 and T6SS2.

First evidence for the presence of pathogenic serogroups of *V. cholerae* in chironomids

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Vibrio cholerae causes the fatal cholera diarrhea and is a natural inhabitant of aquatic ecosystems. Chironomids (Insecta: Diptera, Chironomidae) are the most abundant macro-invertebrate group in freshwater aquatic habitats and estuaries. They undergo a complete metamorphosis of four life stages; three (eggs, larvae and pupae) take place in the water, while the adults emerge into the air. Chironomids are natural reservoirs of *V. cholerae* and we commonly isolate *V. cholerae* from all four life stages of chironomids. However, up until now, only the non-O1/non-O139 serogroups were identified from chironomids. Our aim was to explore the presence of pathogenic strains of *V. cholerae* in chironomids. To obtain this purpose we sampled all four life stages of chironomids from two rivers in Pune, India. In total, we analyzed 173 chironomid samples. The identity of chironomid species was verified using cytochrome oxidase gene sequences. Seven chironomid species were identified. Of them, Chironomus circumdatus was the most abundant (62%). The presence of *V. cholerae* in all chironomid life stages was verified by amplifying the OmpW gene (a gene of an outer membrane protein). Moreover, we were able to amplify wbe and/or wbf genes that detect serogroups O1 or O139, respectively (46% and 13% positive samples, respectively). Cholera toxin subunit A (ctxA) was detected in 49% of the samples. This is the first evidence that pathogenic strains of *V. cholerae* inhabit chironomids. The results of the current study may provide significant novel tools for monitoring and predicting cholera epidemics.

Vibrio ecology in the semi-arid tropics

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In semi-arid tropical north Australia, vibrio illnesses have been reported from seafood consumption and seawater exposure. Most *vibrio* ecology knowledge is from temperate regions and the knowledge gap for the tropics is a problem because there is a growing interest in indigenous-lead tropical aquaculture including oysters. To address this gap we sought to understand key environmental drivers of change in the diversity of vibrio communities and abundance of selected species using high throughput amplicon 16S rRNA sequencing and qPCR. Few 16S rRNA *Vibrio* sequences were recovered and these were only resolved to genus. Another approach using the heat shock protein gene Hsp60 is currently being

assessed as an alternative community level tool for vibrio ecological studies. Using qPCR, *V. parahaemolyticu*s was detected in seawater more frequently and at higher concentrations than *V. vulnificus. V. parahaemolyticus* had a clear positive association with temperature (season) and conductivity, and a negative relationship with dissolved oxygen. In contrast, *V. vulnificus* had a clear positive association with total nitrogen levels, turbidity and conductivity. Prevalence of *V. parahaemolyticus* in warmer waters during the hot wet season may have implications for indigenous sea-based oyster aquaculture. Shellfish safety may define 'best time' to harvest but will this concur with 'right time' to eat from a traditional perspective?

Dynamics of coastal marine microorganisms in response to green tide-derived dissolved organic matter

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An increasing of macroalgae blooms have been observed in many marine environments, seriously affecting the balance of marine ecology. However, the effects of these blooms, especially the dissolved organic matter (DOM) derived from macroalgae, on dynamics of microbial community are still unclear. To identify the responses of heterotrophic bacteria to DOM, we performed microcosm incubation experiments in the laboratory and monitored the microbial community succession of natural seawater using DOM derived from green algae Ulva prolifera. Based on the abundances of culturable, active and total bacteria, we clarified the whole process of bacterial community succession. We found a robust growth of *Vibrio spp*. within short timescales, the relative abundance of which accounted for up to 60% of the active bacteria within 6 h of incubation. In particular, *Vibrio parahaemolyticus* was the most abundant species. In contrast, the genus Donghicola became the dominant group later in the experiment, indicating that this group might be the main degrader of bacteria-derived DOM. Additionally, the DOM addition would reduce the bacterial diversity, and different DOM concentration could influence the bacterial community composition. Meanwhile, the exoenzymatic activities in seawater changed along with the fluctuations of some genera abundance, indicating that the mechanism of DOM utilization by bacteria varies with time. Our study demonstrated the dynamics of bacterial community after DOM influx, shedding new light into a synergistic promoting effect of bacteria in the marine carbon cycle.

Session 2 – Systematics & Evolution

Evolutionary model cluster Divergence of the Emergent Marine Pathogen *Vibrio vulnificus*

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Vibrio vulnificus, an opportunistic pathogen, is the causative agent of a life-threatening septicemia and a rising problem for aquaculture worldwide. The genetic factors that differentiate its clinical and environmental strains remain mostly enigmatic. Furthermore, clinical strains have emerged from every

clade of *V. vulnificus*. In this work we investigated the underlying genomic properties and population dynamics of the *V. vulnificus* species from an evolutionary and ecological point of view. We found evidence that recombination and gene flow between the two largest clusters (C1 and C2) has drastically decreased, to the point where they are borderline different species. Pangenome and phenotypic analyses showed two markedly different lifestyles for these two clusters suggesting a commensal (C2) and bloomer (C1) ecotypes, with differences in carbohydrate utilization, defense systems and chemotaxis among other. Nonetheless, we identified frequent intra- and interspecies exchange of mobile genetic elements providing large genetic diversity in the population (e.g. antibiotic resistance plasmids, novel chromids, or two different and concurrent type-VI secretion systems). Surprisingly, we identified strains from both clusters in the mucosa of aquaculture species. Conclusions. Our results indicate that most *V. vulnificus* clinical are part of two highly divergent clusters with different life styles and pathogen potential. Manmade niches appear to be bringing strains from the two clusters together posing a potential risk of recombination and emergence of novel variants. We propose an evolutionary model of *V. vulnificus* that could be broadly applicable to other pathogenic Vibrios and facultative bacterial pathogens to pursue strategies to prevent their infections and emergence.

Session 3 – Host-microbe Interactions

Anaerobic nitrate reduction divergently governs population expansion of the enteropathogen *Vibrio cholerae*

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To survive and proliferate in the absence of oxygen, many enteric pathogens can undergo anaerobic respiration within the host by using nitrate (NO3-) as an electron acceptor. In these bacteria, NO3- is typically reduced by a nitrate reductase to nitrite (NO2-), a toxic intermediate that is further reduced by a nitrite reductase. However, *Vibrio cholerae*, the intestinal pathogen that causes cholera, lacks a nitrite reductase, leading to NO2- accumulation during nitrate reduction. Thus, *V. cholerae* is thought to be unable to undergo NO3--dependent anaerobic respiration. Here, we show that during hypoxic growth, NO3- reduction in *V. cholerae* divergently affects bacterial fitness in a manner dependent on environmental pH. Remarkably, in alkaline conditions, *V. cholerae* can reduce NO3- to support population growth. Conversely, in acidic conditions, accumulation of NO2- from NO3- reduction simultaneously limits population expansion and preserves cell viability by lowering fermentative acid production. Interestingly, other bacterial species such as Salmonella typhimurium, enterohaemorrhagic Escherichia coli (EHEC) and Citrobacter rodentium also reproduced this pH-dependent response, suggesting that this mechanism might be conserved within enteric pathogens. Our findings explain how a bacterial pathogen can use a single redox reaction to divergently regulate population expansion depending on the fluctuating environmental pH.

Interpersonal Gut Microbiome Variation Drives Susceptibility and Resistance to *Vibrio cholerae*

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The gut microbiome is the resident microbial community of the gastrointestinal tract, and varies greatly as a function of microbial exposure, nutrition, and history of gut environmental insults such as diarrhea, both cholera and non-cholera. The species composition of this community is highly diverse, but how microbial diversity confers resistance or susceptibility to intestinal pathogens is poorly understood. Using transplantation of complete and defined human fecal microbiomes into several mouse model of infection and colonization, we show the ability of human gut microbiomes to resist the establishment of *Vibrio cholerae* colonization varies up to 30-fold across individuals. We identify key microbiome species able shape the chemical environment of the gut by degrading small molecules such as bile acids used by *V. cholerae* to activate the expression of virulence genes, leading to reduced pathogen colonization. We show that the absence of these functions and species in the small intestine permits increased infection loads on a personal microbiome-specific basis, and that such defects in colonization resistance can be rescued by microbial transplantation. These findings suggest new targets for individualized preventative strategies of *V. cholerae* infection through modulating the structure and function of the gut microbiome.

N-terminal autoprocessing and acetylation of Martx Makes-Caterpillars Floppy-like effector is stimulated by ADP-Ribosylation Factor 1 in advance of Golgi fragmentation

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The MARTX toxin is the primary virulence-associated factor of Vibrio vulnificus. The toxin is a composite toxin comprised of multiple cytotoxic effector domains simultaneously delivered to targeted eukaryotic cells. The Makes Caterpillars Floppy-like (MCF) effector domain contributes to lethal *V. vulnificus* gastrointestinal infection in mice. However, the biochemical linkage between the cysteine proteolytic activity of MCF and its cellular effects remains unknown. In this study, we identify the host cell factors that activate in vivo and in vitro MCF autoprocessing as ADP Ribosylation Factor 1 (ARF1) and ADP-ribosylation Factor 3 (ARF3). Autoprocessing activity is enhanced when ARF1 is in its active (GTP-bound) form compared to the inactive (GDP-bound) form. Subsequent to auto-cleavage, MCF is acetylated on its exposed N-terminal glycine residue. Acetylation apparently does not dictate subcellular localization, as MCF is found localized throughout the cell. However, the cleaved form of MCF gains the ability to bind to the specialized lipid phosphatidylinositol 5-phosphate enriched in Golgi and other membranes necessary for endocytic trafficking, suggesting a fraction of MCF may be subcellularly localized. High resolution electron microscopy and fluorescent microscopy show that MCF causes Golgi dispersal resulting in extensive vesiculation. In addition, host mitochondria are disrupted and fragmented. Surprisingly, ARF1 is not itself processed or post-translationally modified by MCF. Further, only catalytically inactive MCF stably associates with ARF1, thus serving as a substrate trap. Our data indicate

that ARF1 is a cross-kingdom activator of MCF, but reveal that MCF mediates cytotoxicity likely by directly targeting another yet to be identified protein. MCF thus is similar to Cholera Toxin and MARTX Effector DmX in its use of ARFs for toxin activation. This study begins to elucidate the biochemical activity of this important domain and gives insight into how it may promote disease progression.

Session 4 – Vibrios and their Viruses

The interplay of phage-host in coral pathogen *Vibrio shiloi* is important for pathogenesis

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Prophage plays an important role in mediating cell death and biofilm formation in various bacterial hosts. However, the interplay of phage-host in coral pathogens remained largely explored. *Vibrio spp*. is commonly found in coral, colonizing the surface, the mucus and the tissue. *Vibrio shiloi* is a pathogen that has been associated with coral disease from geographically distinct global regions. *Vibrio shiloi* SCSIO 43006 was isolated from the gastrovascular cavity of the scleractinian coral Galaxea fascicularis (Hainan island, China). We demonstrated that the injection of *V. shiloi* SCSIO 43006 in the gastrovascular cavity accelerated the bleaching of G. fascicularis in a temperature-dependent way. Genome analysis revealed that *V. shiloi* harbours one prophage carrying novel regulatory and structural genes. Deletion of this prophages greatly increased biofilm formation and also increased bleaching of coral compared to the wild-type strain. The deletion of this prophage activated genes involved with curli production and c-di-GMP synthesis. Specifically, one gene which shows a low similarity with the autophagy protein 16 (ATG16) in this prophage was highly induced at higher temperature and deletion of this gene increased biofilm formation. Further investigation is needed to explore the function of this prophage and the interplay of this prophage and its host in coral bleaching. Collectively, these results showed that prophage in coral pathogen might be important players in increasing pathogenesis of coral by mediating biofilm formation.

Session 1 – Ecology of Vibrios Part II

Vibrio cholerae lifecycle in the Bay of Bengal Estuary: the hotspot of Asiatic cholera

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Toxignic *Vibrio cholerae* has been associated with cholera, a deadly disease affecting millions globally. Although data on the ecology study of *V. cholerae* are monumental, the source and global transmission of the disease remain largely an enigma. Studies over the years have shown *V. cholerae* to be a component of the bacterial community native to the Bay of Bengal estuary. Despite plankton has been proposed to be a reservoir for *V. cholerae*, toxigenic serogroup strains are rarely isolated from surface water by culturing methods. Although molecular and immunochemical methods have been well-established for detecting *V. cholerae*, the culturing methods are considered as the gold standard for detecting the bacterium in actively growing state, a condition essential for initiating outbreaks of cholera. Viable but non-culturable (VBNC) state has been proposed for *V. cholerae* as a survival strategy during the inter-epidemic period. Fluorescent monoclonal antibody assay has been employed to detect non-culturable cells, which are mostly coccoid cells occurring naturally in *V. cholerae* biofilms. Despite biofilm has been shown to be an integral component of the *V. cholerae* aquatic life cycle, most of such VBNC cells in biofilms fail to produce actively growing cells on culture. Results of our decade-long molecular epidemiology and ecology study in the cholera endemic estuarine villages of Bangladesh, combined with the laboratory microcosm experiment data provide evidence of the aquatic lifecycle of *V. cholerae* in water showing that the bacterium remains actively growing round the year and can cause seasonal outbreaks in response to climate factors.

Co-ocurrence patterns of *Vibrio cholerae* and *Escherichia coli* in various environmental settings

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Vibrio cholerae is the etiologic agent of reemerging cholera that is estimated to cause around 2.8 million cases of illness and 91,000 deaths worldwide annually1. The persistence of *V. cholerae* in multiple aquatic environmental reservoirs can be attributed to interspecific strategies such as responsive gene regulation, biofilm formation, etc. Therefore in this study, the interactions of *V. cholerae* with *Escherichia coli (E. coli*), the most commonly used as an indicator for waterborne pathogens including *V. cholerae*2,3, was investigated through evaluating the survival and growth of both bacteria under different temperature, salinity, pH and nutrition deprivation using plate culturing, fluorescence microscopy and real-time polymerase chain reaction (qPCR). Moreover, a special focus was given to the development of viable but non-culturable (VBNC) state in *V. cholerae* that usually fail to grow on culture media but remain metabolically active to persist during unfavorable conditions under survival competition. The interactions between *E. coli* and *V. cholerae* were also tested in real water samples including surface water samples and drinking water samples collected from Nepal. We highlight that *V. cholerae* interacts *E. coli* differently under different water conditions suggesting that bacterium-bacterium interactions influenced by multiple parameters of ambient water would be a contributing mechanism in regulating the proliferation of *V. cholera* and thus help design better bottom-up control practices towards *V. cholera.*

Characterization of *Vibrio spp*. In seawater, fish and bivalves from Norway

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The prevalence of the human pathogenic *vibrio*s correlates with increase in sea surface temperature. Accordingly, the number of *Vibrio* infections has been increasing in Norway in the last few years. The aim of this study was to examine vibrios in marine samples from Norway, for associated antibiotic resistance genes and virulence genes. Filtrated seawater samples (n=20) were incubated on TCBS agar (37˚C, 24-48h). Fish samples (n=60) and batches of marine bivalves (n=16) were pre-enriched in alkaline peptone water + 2%NaCl with or without polymyxine (42˚C, 18h) prior to inoculation on TCBS (37˚C, 24-48h). In total 135 *Vibrio sp*. strains were isolated and identified as *V. alginolyticus* (n=64), *V. metschnikovii* (n=38), *V. anguillarum* (n=24), *V. aestuarianus* (n=7) and *V. cincinnatiensis* (n=2). Phenotypic resistance to two or more agents was observed in 86 isolates and resistance or reduced susceptibility was observed for ampicillin, cefotaxime, ceftazidime, imipenem, oxolinic acid, erythromycin, tobramycin, gentamicin and aztreonam. Sequenced genomes revealed presence of blaCARB, Class C β-lactamase, VarG family subclass B1-like β-lactamase, CatB-related acetyltransferase, tet34 and qnr genes. All V. metschnikovii were α-hemolytic on TSA with 5 % sheep blood or human blood, except five isolates that were β-hemolystic on 5 % sheep blood. In conclusion, all isolates phenotypical susceptible to antibacterial agents recommended for treating of *Vibrio* infections, although genes associated with resistance to tetracyclines and cephalosporins were detected.

Species interactions in a chitin degrading community

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Vibrios are a dominant chitin degrading member of marine microbial communities. Chitin is one of the most abundant biopolymers on the planet and a vital nutrient source for marine microbes. Chitin is collectively degraded by microbial communities. These communities consist of species in three primary metabolic categories: chitin degraders, consumers and cross-feeders. Chitin degraders secrete enzymes that perform the exodigestion of the polymer. They then utilize these degradation products as growth substrates. Consumers do not have the necessary enzymes to degrade chitin themselves but utilize the subunits of chitin that are generated by degraders via extracellular chitin breakdown. Cross-feeders are unable to consume chitin subunits. They rely on excreted metabolites (e.g. Acetate) from the other two types as resources. We are examining how bacterial species adjust their metabolic behaviour as a response to other community members and whether functionally similar types can be replaced with each other without altering functions measured at the community level. To experimentally address these questions, we are using a naturally derived chitin degrading consortia. In this work, we show that species-specific interactions between *Vibrio* degraders and cross-feeders lead to increased chitinase activity in co-cultures compared to monocultures. We are further investigate the interactions between individual *Vibrio* degraders and cross-feeders by quantifying the consequences of their metabolic interactions on single-cell growth rates using a novel microfluidic approach. These experiments provide new insights on how the specificity of interspecies interactions influences metabolic functions at the level of communities.

Session 5 – Genome Biology

Population structure analysis of *Vibrio vulnificus* based on multi locus sequence typing data submitted to PubMLST, 2004-2019

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PubMLST hosts multi locus sequence typing (MLST) and isolate data of dozens of bacteria and few eukaryotes. Since 2004, MLST data had been submitted by researchers from around the globe. Previous publications revealed that *V. vulnificus* are divided into two major subpopulations where one is dominated by human-pathogenic isolates and the other by environmental isolates. The purpose of this study was to examine whether the addition of sequence and isolate data over the past 15 years provided new insights to the population structure of this important pathogen. To date, 574 isolates and 1153 sequence data have been submitted to https://pubmlst.org/vvulnificus/, resulting into 459 sequence types (STs). Of which, 188 isolates were human-pathogenic and the rest (n=386) were isolated from shellfish, eels, and environmental sources. The oldest data was obtained from a US strain isolated in 1975 and the most recent was from 2019. The most common STs in the dataset were ST-8 (*V. vulnificus* biotype 3 from Israel) and ST-6 (eel-pathogenic strains). The vast majority of isolate data was submitted by researchers from China, Germany, Israel, and USA. Phylogenetic analysis of the human-pathogenic strains revealed a distinct subpopulation exclusively predominated by strains form Europe and Israel, while the other subpopulation consisted of strains isolated from Southeast Asia and USA. These findings suggest that the strains from Southeast Asia and North America descended from a common ancestor, while the population from Europe and Israel could have evolved from distinct environmental sources and less likely to be due to migration of populations from one region to another.

Quorum sensing gene regulation: How *Vibrio harveyi* turns on the light

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Bacteria detect and respond to changes in population density using a cell-cell communication system termed quorum sensing. At the heart of the quorum-sensing pathway in *vibrios* is the master regulator LuxR/HapR. These proteins globally regulate hundreds of genes to coordinate group behaviors. We uncovered independent mechanisms of gene regulation by LuxR/HapR proteins that are intimately connected to nucleoid-associated proteins (NAPs). In *Vibrio harveyi*, LuxR activates the transcription of the bioluminescence genes (luxCDABE) >100-fold at high cell density via binding to seven distinct LuxR sites in the lux promoter. At one site, LuxR synergistically binds DNA with a NAP called IHF, which bends the lux promoter DNA and increases lux gene expression 10-fold. At two sites proximal to the -10/-35 sigma-factor binding site, LuxR directly interacts with RNA polymerase via an interaction domain on LuxR that we recently identified. Finally, we show that another NAP called H-NS represses the lux promoter at low cell density. Biochemical and proteomic data show that LuxR remodels the lux promoter by

displacing H-NS to promote transcription activation. Thus, in the absence of H-NS, LuxR is not required for transcription activation of lux. However, bioluminescence production requires LuxR, suggesting that LuxR indirectly controls LuxCDABE post-transcriptionally. These mechanisms of regulation exist at other promoters in *V. harveyi, Vibrio cholerae*, and *Vibrio vulnificus*, indicating that LuxR/HapR proteins play pivotal roles in strict control of group behavior genes.

Genome comparison of *vibrios* causing AHPND in cultured shrimps

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Acute hepatopancreatic Necrosis (AHPND) is a severe disease affecting recently stocked cultured shrimps. It originated in China in 2009 and has spread to many countries in Asia and then to Mexico in 2013. The causative agent are species of the *Vibrio* core group, mainly several strains *of V. parahaemolyticus*, but also strains of *V. owensii* and *V. campbellii*. Several genomes are available from strains isolated from affected shrimps and proven by challenge to be the etiological agents. As with all *vibrios*, they all have two chromosomes, and they differ between strains. MLSA analysis of the strains showed different lineages not related to their geographic origin. Extrachromosomal elements were found in all genomes, most notably one or two large conjugative plasmids. One of these plasmids, pVA1 harbors a 5.5 kbp transposon that contains an operon composed of two genes encoding for a delta endotoxin (PirAB). These toxins are the responsible for the lysis of the epithelial cells of the hepatopancreas. Differences in the plasmid sequences are observed between strains from Asia and those from the Americas; the latter ones have a second transposon not found in most Asian strains. Sometimes a second conjugative plasmid has been observed, with genes that could be related to some virulence mechanisms, but their implication in the disease has not been established.

The Canadian Society of Microbiologist (CSM) Student Oral Competition

Identification of the erythrocyte binding domain of the Flagellar-regulated Hemagglutinin, FrhA, of *Vibrio Cholerae*

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Vibrio cholerae is a Gram-negative bacterium that is the causative agent of cholera. Once ingested, *V. cholerae* colonizes the small intestine where it expresses Cholera Toxin (CT) which causes the characteristic diarrhea associated with the disease. A large flagellar regulated hemagglutinin, FrhA (2251aa), contributes to *V. cholerae* intestinal colonization, and mediates hemagglutination (HA) of red blood cells and epithelial cell binding in vitro. FrhA contains a C-terminal “RTX-like” Type I secretion (T1S) signal, and T1S genes that encode an innermembrane ATPase (FrhB), a membrane fusion protein

(FrhD), and an outer membrane pore (FrhC) have been identified that are required for FrhA secretion and HA. Encoded within the frhDB operon are LapG- and LapD-like proteins that indicate that FrhA surface localization is controlled by cyclic di-GMP levels within the cell. FrhA contains a number of cadherin-like repeats that likely function to extend the protein from the cell surface following Ca2+ binding. FrhA alson contains two domains (RIII_3and RIII_4) with homology to the surface adhesin MpIBP from Marinomonas primoryensis, as well as three additional domains: Ig-like (IGL), sugar-binding (SBD), and unknown function (UKD). Deletion of all five of these domains in *V. cholerae* prevents HA, and removing each domain individually revealed that the RIII_3 domain mediates binding to erythrocytes. MpIBP RIII_3 is a peptide binding domain, suggesting that FrhA RIII_3 is likewise binding to a peptide on the surface of red blood cells.

Horizontally transferred type VI secretion system effector-immunity gene arrays determine life and death in a diverse *Vibrio cholerae* population

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Vibrio cholerae uses its type VI secretion system (T6SS) to inject toxic effectors into neighbouring cells, while immunity proteins specific to each effector protect them from self-intoxication and foreign attacks (1). Due to the large diversity of effector-immunity (EI) gene combinations in *V. cholerae*, T6SS mediated competition has been hypothesized to play an important role in spatially structuring populations of the species (2, 3). Analyzing the genomes of the 17 major lineages of a single *V. cholerae* population, we show that local EI gene diversity is comparable to that of the species' global EI gene pool. Notably, almost all co-occurring lineages possess incompatible EI gene sets, which should lead to mutual killing upon contact. In pairwise co-culture competitions, the vast majority of strains experience reciprocal losses ranging from 20 to almost 100% of cells, in accordance with in-silico predictions. By contrast, the few lineages possessing compatible EI gene combinations encounter negligible reduction. While these strains differ between each other by upwards of 50,000 nucleobases, the outcomes of such competitions are indistinguishable from those using a single clonal lineage. Only in very few cases are individual strains capable of clearly outcompeting other specific isolates. In all of these scenarios, the winning strains possess immunity genes protecting them from all incoming effectors, while losers lack at least one such gene. Our results support a model where the diversity of *V. cholerae* populations is maintained through the creation of a patchwork of microniches by T6SS mediated competitive exclusion.

Novel family of membranal type VI secretion system effectors revealed by comparative genomics

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Type VI secretion system (T6SS) is a protein delivery apparatus found in Gram-negative bacteria. T6SS is often used in interbacterial competition as bacteria deliver toxins, called effectors, into neighboring cells. Antibacterial T6SS effectors appear in bicistronic units adjacent to cognate immunity genes that

protect against self-intoxication. *Vibrio parahaemolyticus*, a marine pathogen that causes acute gastroenteritis, harbors an antibacterial T6SS1 that is found predominantly in pathogenic isolates. Previous reports showed that different *V. parahaemolyticus* isolates carry diverse T6SS1 effector/immunity repertoires. Yet, the identities of T6SS1 effectors in many *V. parahaemolyticus* isolates remain unknown. Here, we used a comparative genomics approach to uncover new T6SS1 effectors. Using this methodology, we searched the genomes of two clinical *V. parahaemolyticus* isolates and identified novel antibacterial T6SS1 effectors. Two of the newly identified effectors shared a homologous C-terminal region containing two transmembrane helices and a conserved motif. Homology searches revealed that these effectors represent a novel, widespread family of T6SS effectors which are found in many pathogenic bacteria. Members of this effector family, which we named Tme (T6SS membranal effector), exerted their toxicity in the periplasm of Gram-negative bacteria and disrupted membrane integrity, likely by forming pores. A conserved family of cognate immunity proteins was also found encoded downstream of Tme effectors. Our findings demonstrate the use of comparative genomics to identify new T6SS effectors, and provide insight into a new family of T6SS-associated, membrane-disrupting toxins.

Vibrio spp. In the South-Eastern Baltic Sea coastal and transitional waters: long term development, present status and model simulations

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First cases of infections of *Vibrio* due to contact with the Baltic Sea water were alerted already in 1978 in Denmark. To date, three *Vibrio* species such as *V. vulnificus, V. cholerae* and *V. parahaemolyticus* have caused the majority of *Vibrio* infection cases in the Baltic Sea region. Regardless of increasing numbers of *Vibrio spp*. infections throughout the years due to warmer waters, in some south-eastern (SE) Baltic Sea countries like Estonia, Latvia, Lithuania and Poland, comprehensive monitoring programs for the occurrence of *Vibrio* in the bathing waters or surveillance systems of *Vibrio* infection cases are not established yet. As a consequence, no knowledge exists about the presence of pathogenic *Vibrio* and potential risks for bathers are unknown. In 2017, *V. cholerae* and *V. vulnificus* were identified for the first time in Lithuanian coastal waters. *V. vulnificus* were found at all coastal bathing waters, except lagoon sites, and vvhA gene varied from 2.8 x 103 to 3.7 x 104 copies L-1 with the highest amounts in sites with average water salinity of 7.1 PSU. Also a long-term *Vibrio spp*. monitoring data of the German Baltic Sea coast was analyzed with a focus on the Bay of Greifswald where higher concentrations of *Vibrio* are observed in comparison to other coastal sites. Several model scenarios were simulated to identify *Vibrio* 'hot-spots' and pathways in the Bay of Greifswald and understand the role of sediment resuspension and increasing water temperature for the proliferation of *Vibrio spp*.

Multifaceted adaptations of vibrios to their molluscan host, the oyster Crassostreae gigas

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The oyster Crassostrea gigas harbors a diversity of *vibrio* populations as part of his normal microbiota. As a host, it is capable to control bacteria by an arsenal of cellular and humoral innate defenses, confining microorganisms to the hemolymph thus preventing the invasion of deeper tissues [1]. The disruption of this immune homeostasis allows the proliferation of disease-causing opportunistic bacteria that severely impact the farming of this species. Notably, in the Pacific Oyster Mortality Syndrome, which affects juvenile oysters during summer, immune suppression by the OsHV-1 virus leads to dysbiosis and fatal proliferation of virulent *Vibrio* species [2]. Nevertheless the mechanisms used by nonvirulent *vibrios* to colonize their healthy host are largely ignored. To explore this question we benefited from virulent and nonvirulent strains of the specie Vibrio splendidus isolated from the oyster in absence of the OsHV-1-induced dysbiosis [3]. Two strategies were evidenced according to strain virulence. In virulent strains, we found that a MARTX type toxin and a Type 6 Secretion System allow *Vibrio splendidus* to suppress host defenses and engage into inter- bacterial competition enabling successful host colonization. In nonvirulent strains, we observed a shrinking of LPS linked to a differential glycosylation of the oligosaccharide core and O-antigen. This suggests that adaptations towards virulence attenuation and less immunogenic surface properties are used by commensal *vibrios* whereas active mechanisms to repress immune defenses and fight bacterial competitors are used by virulent *vibrios* to colonize oysters.

A pathogenic biotype of *vibrio coralliiyticus* uses antibiotic-production to overcome the protective coral microflora

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Coral disease outbreaks can have a devastating effect on reefs by directly killing the coral animal, which further degrades threatened reef systems. The healthy microflora present on corals is speculated to prevent colonization by bacterial pathogens such as *Vibrio coralliilyticus*. However, there is little causative evidence for the protective capabilities of the resident microflora or the mechanisms by which pathogens overcome these defenses. Manipulative experiments with the Hawaiian rice coral, Montipora capitata, demonstrated that disruption of the coral microflora with antibiotic pre-treatment allows for less or seemingly non-virulent *V. coralliilyticus* strains to infect. Infection rates by *V. coralliilyticus* increased by 40-100% following treatment with ampicillin and spectinomycin for 24 h prior to pathogen exposure. Strain OCN008 was originally isolated based on antibacterial activity, which was later found to be caused by the broad-spectrum antibiotic andrimid. The andrimid biosynthesis cluster (adm) was not found in other known coral pathogens, and deletion of the adm gene cluster (adm mutant) reduced the infection rate of OCN008 by 60% as well as its ability to colonize corals. Pre-treating coral fragments with purified andrimid or other antibiotics before inoculation with the adm mutant complemented the mutant. Furthermore, treatment with andrimid reduced the culturable coral microflora without any observable negative effects on the coral. These results demonstrate that strain OCN008 uses andrimid production as a virulence factor to induce dysbacteriosis of the coral microflora, thus facilitating pathogen colonization and further validating the protective role of the microflora in coral disease.

Influence of *Vibrio spp*., temperature, reproductive development, and stocking density on pacific oyster (Crassostrea gigas) summer mortality in Baynes Sound, British Columbia

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Cultured Pacific oysters (Crassostrea gigas) in Baynes Sound, British Columbia, have experienced summer mass mortality events in recent years with cumulative mortalities exceeding 90% at some sites in 2015 and 2016. In 2017, we isolated *Vibrio spp*. from oysters and putatively identified potentially pathogenic species, based on recA gene sequencing; among the 163 isolates, *V. aestuarianus* and *V. harveyi* were well represented. The objective of the present study was to identify factors influencing the onset of a mortality event in juvenile Pacific oysters during the summer of 2018. We recorded mortality, growth, gonad development, temperature, turbidity, dissolved oxygen, chlorophyll-a, plankton assemblages, and bacterial community composition. Our study site contained four replicate trays of four stocking densities: 150, 300, 450, and 600 oysters/tray. Mortality was first observed on July 30, which coincided with a marine heatwave. *Vibrio aestuarianus* and the proportion of *Vibrio spp*., quantified using qPCR and community 16S rRNA gene sequencing, respectively, increased with observed mortality rate. Mortality rates were at their highest on August 12, and we observed systemic mixed microbial infections in histological cross sections of oysters that otherwise appeared healthy. The final cumulative mortalities ranged from 34 to 75%, with the highest density trays having significantly lower mortality than the lowest density trays. Significant density-dependent effects were also observed for oyster size and gonad development. The long-term persistent ocean warming and increased frequency of marine heatwaves associated with climate change are likely contributing to the emergence of summer mortality and pathogenic Vibrio spp. in Baynes Sound.

The antibacterial and anti-eukaryotic type V1 secretion system MIX-effector repertoire in *Vibrionaceae*

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The *Vibrionaceae* family is widespread in aquatic ecosystems and includes pathogenic bacteria. Many members of this family employ type VI secretion system (T6SS), a toxin injection apparatus that targets neighboring bacteria or eukaryotes, thus mediating interbacterial competition or virulence, respectively. Antibacterial effectors can cause self-intoxication, and are therefore encoded together with a cognate immunity protein. Previously, an N-terminal domain named MIX (Marker for type sIX effectors) was defined as a marker for T6SS effectors. MIX-effectors are widespread, polymorphic toxins that carry various C-terminal toxin domains. In this study, we used a computational approach and set out to identify the Vibrionaceae MIX-effectors arsenal and analyze the various toxin domains that they carry.

We identified ~3000 MIX-effectors in available *Vibrionaceae* genomes, and grouped them into clusters based on their predicted C-terminal toxin domains. We then classified MIX-effectors as either antibacterial or anti-eukaryotic based on the presence or absence of adjacent putative immunity genes, respectively. Using this approach, we uncovered a diverse set of MIX-effectors displaying various antibacterial toxic activities that target vital cell components such as cell membranes, peptidoglycan, and nucleic acids. Moreover, we discovered MIX-effectors with anti-eukaryotic functions that are predicted to play a role in virulence. Interestingly, these effectors are encoded by strains that harbor a wide arsenal of MIX-effectors, which we named "professional MIXologists". Our findings suggest that certain *Vibrionaceae* species adapted their antibacterial T6SS to also mediate interactions with eukaryotic hosts to enhance their fitness.

SmcR, master regulator of quorum sensing in *Vibrio vulnificus*, requires two distinct approaches to transcriptional activation

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Quorum sensing is a density-dependent cell-cell communication system used by bacteria to regulate group behaviors. Our lab uses *vibrios* as model organisms to study quorum sensing. *Vibrio vulnificus* utilizes SmcR as the master regulator of quorum sensing, a homologue to the well-studied LuxR/HapR proteins in *Vibrio harveyi* and *Vibrio cholerae*, respectively. These transcriptional regulators are unique members of the TetR family of transcription factors as they are capable of both activating and repressing genes. However, it has remained unclear how SmcR binding differentiates between promoters of activated and repressed genes in vivo and how protein interactions are involved. Using biochemical and structural approaches to analyze mutants of SmcR, we investigated differences in SmcR-DNA interactions and SmcR-protein interactions that drive activation-defective phenotypes. Our results indicate two categories for defects in transcription activation of these mutant proteins: 1) disruption of SmcR-protein interactions (e.g., RNA polymerase), and 2) disruption of SmcR DNA binding at a subset of SmcR binding sites in the promoter (in addition to those proximal to RNA polymerase). Using X-ray crystallography, we solved structures of SmcR mutants from both categories. An activation-deficient mutant of SmcR with a substitution in the helix-turn-helix displayed a measurable shift in this domain, which disrupted DNA binding and transcriptional activation at some promoters. Conversely, SmcR mutants with substitutions in the protein interaction domain did not exhibit any shifts in secondary structure. We conclude that SmcR requires DNA binding activity at multiple sites in the promoter as well as protein interactions for full transcriptional activation.

Type III Secretion System Gene Expression and Virulence is Regulated by a DNA Cruciform Structure at the exsA promoter in *Vibrio parahaemolyticus*

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Bacterial pathogens temporally upregulate virulence gene expression to promote infection, subverting host defenses and leading to disease (1,2). In the case of pandemic *Vibrio parahaemolyticus* (*Vp*) strains, two Type III Secretion Systems (T3SS), along with a variety of toxins contribute to acute enteric disease in humans. Previously, we reported that *Vp* derepresses the genetic expression of its T3SS-1 master transcriptional regulator, ExsA, by encoding a HlyU regulator during infection (3). Specifically, HlyU dimers act as DNA binding transcriptional regulators to relieve exsA gene silencing mediated by the histone-like protein H-NS (4). We have shown that HlyU binds a 56-bp DNA region within the exsA promoter containing an inverted repeat sequence separated by a 14-bp A/T rich palindromic sequence. In silico modelling lead us to hypothesize that a 4-way stem loop DNA cruciform structure forms at the inverted repeat, and that this DNA superstructure plays a key role in T3SS-1 gene regulation. T7 endonuclease I digestion, which cleaves at 4-way DNA cruciforms, suggested that the exsA promoter DNA forms a 4-way DNA superstructure. Moreover, the DNA cruciform superstructure was restriction mapped to the inverted repeat sequences and cruciform formation was strongly dependent on supercoiled DNA. Further, in situ real time transcription assays showed that specific mutations designed to disrupt stable cruciform formation reduced exsA promoter activity compared to the native exsA promoter. Overall, the data highlight a biologically relevant DNA cruciform superstructure within *Vp* that serves to regulate virulence gene expression with the cooperation of HlyU DNA binding.

Session 6 – Vibrio Challenges to the Seafood Industry

Emerging pathogens and traditional food impacts: *Vibrio cholerae* and herring eggs

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[1]Island Health, [2]First Nations Health Authority BC, [3]First Nations Representative

The first known outbreak of non-toxigenic *Vibrio cholerae* in British Columbia occurred in March 2018 on Vancouver Island. The illnesses were associated with the consumption of an important traditional seafood for many First Nations in BC: herring eggs harvested through spawn on kelp; it is suggested that climate change had a role to play. This keynote will focus on the collaborative approach between public health agencies, First Nations communities and other federal and provincial stakeholders that worked together during the outbreak investigation, and to mitigate any ongoing health risks, and to understand this emerging pathogen that agencies were not equipped or prepared for. Presenters will share the story of how this outbreak impacted public health and communities where traditional food sources provide important nutrition, culture and economic value. Conference participants are welcomed to share ideas and opportunities to assist BC in further understanding this emerging issue, and most importantly, to assist BC First Nations in regaining confidence in this traditional food source.

A new high throughput sequencing assay for characterizing the diversity of natural *Vibrio* communities and its applications during a Pacific oyster mortality event

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The *Vibrio* genus is notable for including pathogens of marine animals and humans, yet characterisation of *Vibrio* diversity using 16S rRNA sequencing methods is often constrained by poor resolution beyond the genus level. Here, a new high throughput sequencing approach targeting the heat shock protein (hsp60) as a phylogenetic marker was developed to more precisely discriminate members of the *Vibrio* genus in environmental samples. The utility of this new assay was tested using a mock community constructed from known dilutions of *Vibrio* isolates. Relative to standard 16S rRNA and Vibrio-specific 16S rRNA sequencing assays, the hsp60 assay delivered high levels of fidelity with the mock community composition at the species level. This assay was subsequently applied to characterise Vibrio community composition in seawater and delivered substantially improved taxonomic resolution of Vibrio species compared to 16S rRNA analysis. Finally, this assay was applied to examine patterns in the Vibrio community during a Pacific oyster mortality event. In these DNA samples, efficacy of the hsp60 assay was dependent on *Vibrio* abundance, but identified species level *Vibrio* community shifts prior to disease onset in oysters, pinpointing *Vibrio harveyi* as a putative pathogen. Given that shifts in the *Vibrio* community often precede, cause and follow disease onset in numerous marine organisms, there is a need for an accurate high-throughput assay for defining *Vibrio* community composition in natural samples. This *Vibrio*-specific hsp60 assay offers the potential for precise high throughput characterisation of *Vibrio* diversity, providing an enhanced platform for dissecting *Vibrio* dynamics in the environment.

Harmonized diagnostic practice to promote prudent use of antimicrobials in aquaculture: ongoing *Vibrio spp*. antibiogram research initiatives

Sandrine Baron

Anses-UMBA

Promoting prudent use of antimicrobials is nowadays the leitmotiv of UN organisations such as FAO, OIE and WHO. Their main fear is to get back to clinical practice from one century ago. In the meanwhile, in many aquaculture-leading countries, antibiotic usage, in either humans or food-producing animals, is not regulated. Several *Vibrio* species are considered as fish pathogens. Some *Vibrios* are causing important economic loss to the fish and shrimp industries. However, treatments are available. Unfortunately, in absence of effective diagnostic tools, veterinarians have to implement hazardous empirical antimicrobial treatments. In a recent review, Egan & Smith (2018), examined the published antimicrobial susceptibility studies of *Vibrio spp*. isolated from diseased fish. Only 9% of the 207 published studies had been using standardized protocols, interpreted through internationally harmonized criteria and reported accurately their raw data. Consequently, for the vast majority of the studies, serious methodologic weaknesses made the results unusable. It is true that susceptibility testing raw data collection is far for being enough for clinician to be able to predict a treatment outcome: pharmacological data and empiric clinical records are also necessary. Nevertheless, delineating bacterial populations that had aquired a resistance mechanism to the treatment from the bacterial population that hadn’t, is a key step to interprete antibiogrammes. That means setting threshold, called epidemiological cut-off values, by collecting harmonized data from at least 3 laboratories and 30 strains. Recent international initiatives are on going to fill that data gap. Two multicountry European initiaves on

several Vibrio species to be presented.

Lactic acid bacteria and *Vibrio parahaemolyticus*: growth inhibition and virulence attenuation – Application to shellfish products

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V. parahaemolyticus is responsible for disease outbreaks following consumption of raw or undercooked shellfish. The aim of this project is to propose new strategies to limit contamination of seafood products (shellfish) by *V. parahaemolyticus* and thus, human infections. A collection of lactic bacteria (LAB) was selected to evaluate their capacity to inhibit growth and/or attenuate virulence of pathogenic *Vp* strains. Over 3000 marine bacteria (60 genera, 900 lactic bacteria, 600 hydrothermal bacteria and others) were first screened in vitro for their capacity to inhibit the growth of *V. parahaemolyticus* LMG2850. Co-culture assays of lactic bacteria and *Vp* LMG2850 allowed to select the best candidate LABs and to test them against 11 *Vp* strains with various virulence genetic profiles. The impact of the selected LABs on the virulence of the 11 *Vp* strains was evaluated in vivo by co-infection of the nematode Caenorhabditis elegans. WGS of LABs and *V. parahaemolyticus* has been performed. The LAB and *Vp* LMG2850 co-culture assays allow to select 38 inhibiting LABs belonging to different genera, i.e. Lactobacillus, Lactococcus, Carnobacterium and Weissella. The following assays with 10 LABs and 11 *V. parahaemolyticus* strains showed variable inhibition of *Vp* growth. This inhibition was mainly due to acidification but could also to due to another mechanism in one of the LAB/*Vp* assay (bacteriocins). This latter observation has to be confirmed. *Vibrio parahaemolyticus* virulence expression in vivo with the *C. elegans* model varied among strains. The effect of the LABs on *Vp* virulence by co-infection in the *C. elegans* model is being studied. If any effect, LAB and *Vp* genome will be analysed to investigate factors involved in virulence attenuation and first in vivo assays will be performed in oysters.

Ecological and Genomic Studies of *Vibrio parahaemolyticus* to Reduce illnesses and Improve Management in the Oyster Industry of New England

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Vibrio parahaemolyticus is the leading cause of seafood-borne morbidity in the US, including New England where shellfish-borne illnesses had been rare until 8 years ago with the onset of significant warming of coastal waters. Most confirmed illnesses from regional shellfish have been cause by one strain, ST36, even though it has rarely been detected directly from New England coastal areas. States have decreased illnesses since their peak in 2013 through effective management strategies, though

illnesses and outbreaks continue to occur. Recent monitoring of total *V. parahaemolyticus* populations in oysters, along with environmental and ecosystem variables, has lead to a better understanding of its ecology to inform characterization of regional risk conditions and management practices. In the Great Bay estuary of New Hampshire, in-depth sampling and ecosystem monitoring over 10 years has lead to the development of multi-parameter models that can accurately track the seasonality, dispersion, and overall trends of total *V. parahaemolyticus* populations. Recent efforts have also lead to the development and field assessment of detection methods that target ST36 and other less common regional clinical strains, in concert with studies on shellfish aquaculture practices that may increase public health risks. The ST36 lineage is noteworthy because it has persisted in the Atlantic Ocean since it was introduced. Genomic analysis indicates dynamic phage-host interactions played a role during lineage diversification that preceded invasion into the Atlantic Ocean. Continued studies are focused on phage-host ecology and applying ecosystem models to track pathogenic strains to further our understanding of regional risk conditions.

Advanced molecular approaches for development of rapid molecular diagnostics assays for virulent *Vibrios*

Rachel Nobel

UNC Chapel Hill

Vibrio parahaemolyticus and *V. vulnificus* are abundant members of native microbial assemblages in coastal waters estuaries, and are found in molluscan shellfish, such as oysters, which can concentrate the bacteria up to 100-fold as compared to the water column. Though these species are predominantly environmental, some strains have the capacity to infect human hosts and cause outbreaks of seafood-related gastroenteritis (primarily from raw oyster consumption) and wound infections (primarily from water contact). We have analyzed DNA sequence data to generate information on the genes and non-coding regions that are related to virulence using motif fingerprinting approaches to identify improved molecular targets. We are also conducting work to identify the relationship of these virulence-related sequences to environmental parameters in the hope of developing predictive models that are capable of specifically predicting heightened virulence and therefore risk of infection through QMRA approaches. The motif fingerprinting analysis revealed groups of protein motifs that were associated with the pathogenic strain type ST36 and a large group of clinical strains isolated from human stool for *V. parahaemolyticus*. A subset of the stool and ST36-associated protein motifs were selected for further analysis and the motif sequences were found in genes with a variety of functions, including transposases, secretion system components and effectors, and hypothetical proteins. Molecular diagnostic tests that are specific for virulence will assist in developing better estimations of risk associated with different types of infections, and will assist water quality and aquaculture managers in improved practices to protect public health.

Session 7 – *Vibrio* physiology

Transcriptional regulation by σ factor phosphorylation controls polymyxin resistance in *Vibrio parahaemolyticus*

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In order to survive in changing environments, it is essential for cells to be able to sense their surroundings, respond to extracellular stresses, and adapt gene expression accordingly. A major form of bacterial transcriptional regulation occurs by the exchange of the primary σ factor of the RNA-polymerase with alternative ECF σ factors, which generally are retained in an inactive state by sequestration into σ/anti-σ factor complexes. Using *Vibrio parahaemolyticus* as model organism, we report a novel mechanism of transcriptional regulation, which instead relies on intrinsically inactive ECF σ factors that rely on σ factor phosphorylation for interaction with RNA-polymerase. Particularly, we show that polymyxin stress of *V. parahaemolyticus* activates the threonine kinase PknT. PknT then phosphorylates the σ factor EcfP, resulting in EcfP activation and expression of an essential polymyxin resistance regulon. EcfP phosphorylation occurs at a highly conserved threonine residue, Thr63, positioned within a divergent region in σ2.2 helix. EcfP is intrinsically inactive and unable to bind RNA-polymerase due to the absence of a negatively charged DAED-motif in this region. Phosphorylation at residue Thr63 mimics this negative charge and licenses EcfP for interaction with RNA-polymerase and activation of target gene expression. Transcriptional regulation by σ-phosphorylation is likely a widespread mechanism in bacteria, presenting a new paradigm in transcriptional regulation. Furthermore, we have identified a new sensing and signaling mechanism that regulates polymyxin resistance in *V. parahaemolyticus*.

A dual function sRNA switching the flux of alanine into carbon and nitrogen metabolism in *Vibrio alginolyticus*

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Many small RNAs (sRNA) were reported to play important roles in regulation of carbon metabolism either by base-pairing the targeted mRNAs with the assistant of RNA chaperon protein Hfq, or by sequestering the RNA binding protein CsrA. In this study, we identified a new Csr sRNA named CsrB4 in the fish pathogen *Vibrio alginolyticus* ZJ-T, in addition to three Csr sRNAs which are homologs of CsrB, CsrC and CsrD in *Vibrio cholerae*. It is conserved in the *vibrio* species but absent in the genome of *Vibrio cholerae*. Its expression was induced by amonium and glucose but repressed in LB medium. It is able to rescue the phenotype of CsrB/CsrC/CsrD mutation in *V. cholerae*, suggesting its ability to sequester CsrA. Surprisingly, loss of Hfq decreased its expression by ten times, indicating its dependance on Hfq. Deletion of CsrB4 caused a prolonged lag phase when it was grown in M63 plus glucose and alanine, indicating its involvement in the alanine metabolism. EMSA and sRNA-mRNA interaction alalysis showed

that CsrB4 is able to bind an 11nt seed sequence in the vicinity of the start codon of ald which encodes alanine dehydrogenase with the assistant of Hfq, leading to the inhibition of ald expression. In addition, multiple-omics analysis showed that csrB4 have significant impact on the glycolysis and TCA cycle. These results suggested that CsrB4 may function as a "regulating switch" that controls the alanine flux into carbon or nitrogen metabolism in *V.alginolyticus*.

Storage compound consumption decreases population lag-time by increasing heterogeneity among *Vibrio natriegens* cells exiting nutrient starvation

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Nutrient availability is dynamic over the spatiotemporal scales experienced by individual bacteria in marine ecosystems. When cell division is halted by nutrient starvation, bacteria can enhance their survival or future reproduction by altering their biomass composition and synthesizing storage compounds from the non-limiting nutrients available to them. In this study, we examined how individual cells and populations of *Vibrio natriegens* metabolize storage compounds during starvation and how this alters their ability to regrow when nutrients return. *V. natriegens* synthesizes the carbon storage compound polyhydroxybutyrate (PHB) despite halted cell division during phosphorus starvation. Phosphorus-starved cells hoard PHB to nearly 40% of their biomass and increase in size compared to cells in steady-state growth and carbon-starved conditions. Growth resumption in nutrient replete conditions is much slower after phosphorous starvation than after carbon starvation, with little variability among individual cells in both conditions. PHB synthase mutants do not accumulate measurable amounts of the storage compound after phosphorus starvation and have no measurable growth resumption defects in nutrient replete conditions. However, hoarded PHB can support up to two divisions per cell during a subsequent period of carbon starvation. This PHB-driven reproduction during carbon starvation increases the variability of growth resumption among cells when nutrient replete conditions return. Currently, we are examining if PHB consumption enhances the biosynthesis of phosphorous-rich ribosomes which leads to faster growth resumption. This study demonstrates that PHB metabolism can enhance the growth resumption of a population during certain nutrient fluctuations, but it does not benefit all individual cells equally.

Session 8 – *Vibrio* Secretion Systems

A modular *Vibrio* effector with a Dnase domain and a maker for type VI secretion system substrates

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Bacteria deliver toxic effectors via type VI secretion systems (T6SSs) to dominate competitors, but the identity and function of many effectors remain unknown. We identified a *Vibrio parahaemolyticus*

antibacterial T6SS effector that contains a previously undescribed, widespread DNase toxin domain that we call PoNe (Polymorphic Nuclease effector). PoNe belongs to a diverse superfamily of PD-(D/E)xK phosphodiesterases, and is associated with several bacterial toxin delivery systems including type V, type VI, and type VII. PoNe toxicity is antagonized by cognate immunity proteins (PoNi) containing DUF1911 and DUF1910 domains. In addition to PoNe, the effector contains a domain of unknown function which we named FIX (Found in type sIX effector). FIX domains are found N-terminal to various known or putative toxin domains, and are genetically and functionally linked to the T6SS. We propose that FIX sequences can be used to identify T6SS effector candidates with potentially novel toxin domains. These findings underline the modular nature of bacterial effectors harboring delivery or marker domains specific to a secretion system, that are fused to interchangeable toxins.

Orphan Immunity Proteins in Vibrio cholerae Confer Protection Against Ex-Kin Effectors

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Vibrio cholerae, the causative agent of cholera, is an accidental pathogen that thrives in brackish coastal waters. All *V. cholerae* isolates for which genomic information is available encode a contact-dependent type VI secretion system (T6SS), a harpoon-like complex employed to export proteinaceous effectors that kill neighboring prokaryotic and eukaryotic competitors. The main T6SS components are encoded in three genetic loci (a main cluster and two auxiliary clusters, Aux1 and Aux2), each terminating in a modular effector-immunity protein pair. Effector-immunity pairs at each locus are highly diverse among *V. cholerae* strains. Due to this diversity, a predator cell's effector set confers both inter- and intraspecies killing capacity. Kin cells are protected by cognate immunity proteins, allowing a strain to generate a niche and clonally colonize both aquatic reservoirs and the host gut. We recently provided phylogenetic support for the acquisition of new effector-immunity pairs by horizontal gene transfer, as *V. cholerae* effector sets do not align with the evolutionary lineage of *V. cholerae* strains. Analysis of T6SS loci in *V. cholerae* strains shows that when a new effector-immunity pair recombines into the chromosome, the previous effector gene is displaced. In several instances, however, the previous immunity gene is retained on the chromosome directly downstream of the newly acquired effector-immunity pair. This indicates a non-canonical recombination mechanism and a competitive advantage to immunity gene retention. We hypothesize that the orphan immunity genes function as a type of memory, lending protection against attacks from ex-kin cells. Some orphan immunity arrays contain as many as four different immunity cassettes in environmental *V. cholerae* strains, but pandemic strains of *V. cholerae* harbor a single orphan immunity gene at the Aux1 cluster. This simplified case allows us to investigate recombination events leading to orphan retention and the competitive advantage it lends. Here we show that the Aux1 effector likely originated from an environmental *Vibrio* species. Recombination into the *V. cholerae* genome likely took place in a highly-conserved region of the tap-1 adaptor gene directly upstream for the effector and a region of weak homology near the C-terminal end of the displaced effector gene, leading to the integration of a new effector-immunity pair and maintenance of the previous immunity gene. Further, we demonstrate that the retained immunity gene can neutralize the killing capacity of its ancestral cognate effector protein. This study highlights a mechanism of new effector acquisition that allows a cell to be at odds with its surrounding kin without

becoming vulnerable to their attacks.

Poster Presentations

Session 1 – Ecology of *Vibrios*

Quantification and predication of *Vibrio cholerae* non-O1/non-O139 in bathing waters

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Vibrio cholerae non-O1/non-O139 are natural inhabitants of aquatic ecosystems causing a variety of diseases, such as mild gastrointestinal infections, ear, wound and bloodstream infections or necrotizing fasciitis, with harmful impact on very young, elderly or weakened individuals. In the past two decades an increase in *V. cholerae* non-O1/non-O139 associated infections have been documented in the northern hemisphere, attributed to global warming. In the past years, several cases of *V. cholerae* non-O1/non-O139 infections have been documented in Austria with a local history specifically associated with bathing activities including two cases with fatal outcome. The aim of this study is to monitor the prevalence and abundance of *V. cholerae* non-O1/non-O139 at selected bathing sites in Eastern Austria along spatiotemporal environmental gradients. Solid quantitative data are obtained by culture-based and culture-independent (CARD-FISH/SPC and qPCR) methods, recently developed in our laboratory. Based on these data, combined with environmental information, a prediction model for the prevalence, spread and abundance of *V. cholerae* non-O1/non-O139 in bathing waters is developed. On the one hand, this provides important information for national health authorities concerning the threat of *V. cholerae* non-O1/non-O139 infections in bathing waters. On the other hand, the found correlations and models may also be transferred to other geographic regions.

Vibrio cincinnatiens: Phenotypic and Genotypic Characterization of Veterinary Isolates

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Vibrio cincinnatiensis is a rare human pathogen. Investigations of miscarriages of domestic animals performed by an official veterinary laboratory in Saxony revealed the presence of *V. cincinnatiensis* in

abortion material. We studied nine isolates from abortions of pigs, cattle and horse and one isolate obtained from a goose. A human clinical isolate recovered from a case of enteritis and assigned to the species *V. cincinnatiensis* was also included. All strains were characterized for biochemical and phenotypic traits and their genomes were determined. The biochemical properties of all isolates showed some variable reactions. Only one strain possessed a very weak hemolytic activity against sheep erythrocytes. Serum resistance was intermediate in some strains and few antibiotic resistance phenotypes were observed. Average nucleotide identity (ANI) as a tool to compare genetic relatedness confirmed that all veterinary isolates are closely related to reference strain NCTC12012, a clinical human isolate (ANI values >98 %). An only exception was the human isolate (ANI value 88%). The bioinformatic analysis indicated a common set of potential virulence factors in all isolates. Isolation of *Vibrio cincinnatiensis* from marine environments in Germany has not been reported, therefore the source of the strains is unclear. As the veterinary isolates are closely related to the human pathogenic strain NCTC12012, a zoonotic potential seems possible. The "One Health" concept acknowledges that human, animal, and environmental health is interrelated. Further research should aim to identify reservoirs, sources, and ways of transmission of this species to determine a possible role as zoonotic agent.

Antimicrobial resistance properties within a *Vibrio cholerae* non-O1/non-139 population from a large Austrian lake

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Vibrio cholerae non-O1/non-O139 has received increasing attention in recent literature and by public health authorities due to the rising number of bathing-water associated infections. Here, we investigated the antimicrobial resistance properties in a *V. cholerae* non-O1/non-O139 population from a large Austrian lake intensively used for recreation and in clinical isolates from infections acquired in this lake. Eighty-two environmental isolates representing the genetic diversity of *V.cholerae* in the lake and 9 clinical isolates were analyzed for their phenotypic antimicrobial susceptibility. The genomes of 46 environmental and eight clinical strains were screened for the presence of known antimicrobial resistance traits. Overall, antimicrobial susceptibility of the *V.cholerae* population was high. The environmental strains were susceptible to most of the tested antibiotics, except sulfonamides, streptomycin and ampicillin. Clinical isolates partly showed additional resistance to amoxicillin-clavulanic acid. Genome analysis showed that several multidrug efflux systems and regulators as well as resistance-related mutations in the DNA gyrase and topoisomerase IV were present in all isolates. Six isolates additionally carried a determinant of phenicol resistance, while the nine sequenced ampicillin resistant isolates carried beta-lactamase genes that were concentrated in specific phylogenetic clades. For all isolates, no genes conferring resistance to aminoglycosides, macrolides and sulfonamides were detected. The observed discrepancies between the phenotypic and genome-based antimicrobial resistance assessment show that for *V.cholerae* non-O1/non-O139, resistance databases are currently

inappropriate. The widespread presence of acquired resistances against sulfonamides and streptomycin indicates a continuous selective pressure from these antibiotic classes, inferring consequences for assessing antibiotic input into the lake.

Survival Strategies in *Vibrio toranzoniae*

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Vibrio toranzoniae is a marine bacterium belonging to the Splendidus clade, originally isolated from healthy clams in Galicia (Spain). Further isolation of the species indicated a geographical and host distribution wider than expected. To survive in the environment, bacteria of the genus *Vibrio* present different adaptative strategies. Thus, adhesion to surfaces and biofilm formation play an important role in colonization and bacterial survival, with the flagellum as a key element as initiator agent in both adherence and biofilm formation. In this work, we studied the capacity of adherence to chitin of three strains of *V. toranzoniae* with different host and geographic origin. The strains included were the type strain CECT 7225T, isolated from clams in Galicia; the strain R17, isolated from red conger eel in Chile, and the non-motile strain 96-376, isolated from seawater in Valencia. In addition, a biofilm formation study was carried out, which included the Chilean isolate R17, its mutant for the master regulator of the flagellum, the gene flrA, and the non-motile strain 96-376. Crystal violet dye was used for the detection and quantification of biofilm. The study of adherence to chitin revealed a similar adhesion capacity in the three strains, presenting lower values than those described in other *vibrios*, such as *V. vulnificus*, but similar to other species of the genus like *V. splendidus*. The biofilm formation study showed the ability of R17 to form biofilm, in greater quantity than the mutated strain, which highlights the key role of the flagellum in the biofilm formation.

Occurrence of *Vibrio parahaemolyticus, Vibrio vulnificus*, and *Vibrio cholerae* in the Urbanized Guajara Bay, Amazonia, Brazil

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Little information is available on the presence of *V. cholerae (VC), V. parahaemolyticus (VP)* and *V. vulnificus (VV*) in the Amazonian region where the last cholera epidemic occurred in the early 1990s. The main objective of this study was to investigate occurrence of these vibrios in the Guajará Bay, an important estuary along the Amazonian coast where Belém, the largest city of the Amazonia is located. Water and zooplankton samples were collected in September, 2017, at five stations in the estuary along

a salinity gradient, from 0 to 20‰. Temperature of the water was ca. 29°C. Enumeration of the three *vibrio* species was accomplished using APW enrichment and MPN-real-time PCR analysis. APW broths were spread onto CHROMagar™ *Vibrio* and presumptive *Vibrio c*olonies were isolated for molecular identification. The total number of VC and VP varied from ca. 10 MPN/L to 110 MPN/L, and of VV from 4 MPN/L to 46 MPN/L. Highest levels were recorded for VC and VV when the salinity was ca. 2-4.6 ‰, and ca. 4.6 ‰, respectively. No pattern was observed for VP that could be correlated with salinity. The number of potentially pathogenic VP tdh+ and VP trh2+ varied from ca. 1 MPN/L to 4 MPN/L, and from 1 MPN/L to 2 MPN/L, respectively. The three vibrios were detected on the copepods (Acartia tonsa) and on Cirriped nauplii, depending on the sites and the salinity. Of 109 Vibrio strains isolated at the five stations included in this study, 67 were identified as *V. cholerae*, 27 *V. parahaemolyticus*, and 15 *V. vulnificus*. The vibrio species were widespread through the large salinity gradient of the Guajará bay, showing its relevance as a potential reservoir of these bacteria.

Toxicity comparison of ethidium monoazide and propidium monoazide treatment for the differentiation of viable *Vibrio* cells by real-time PCR

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Ethidium monoazide (EMA) and propidium monoazide (PMA) toxicity was compared for several *Vibrio* species, including reference strains and strains isolated from seafood, in order to develop a qPCR to distinguish between viable and dead cells. Results showed that PMA had no antimicrobial effect, while EMA was toxic for *Vibrio cholerae* and *Vibrio vulnificus* strains.

Epidemiology of non-O1/non-O139 *Vibrio cholerae* in New Zealand: An underappreciated cause of gastrointestinal illness in the community

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The surveillance of *Vibrio* infections in New Zealand is restricted to those caused by toxigenic *Vibrio cholerae* (cholera) and *V. parahaemolyticus*. Isolates identified in clinical laboratories as *V. cholerae* are referred to ESR where they are tested for the presence of the cholera toxin gene (ctxA) and O-antigens O1 and O139. During the period 2002 – 2018, 140 isolates were tested and confirmed as *V. cholerae* but only 13 were ctxA positive. The remainder (n=127) were confirmed as non-O1/non-O139 *V. cholerae*. These were reported to New Zealand's public health surveillance system as 'not a case', resulting in limited additional epidemiological information being collected. The majority (84%) of these isolates came from faecal specimens suggestive of gastrointestinal illness, followed by ear swabs (8%), blood (3%), and site not specified (5%). While cases occurred throughout the year, more were reported over the summer months (December to April), consistent with seasonal trends reported in other countries. Cases were more likely to be male and 45 – 65 years of age. Data on risk factors were sparse. The

available information indicated shellfish consumption or overseas travel were important. The estimated annual incidence ranged between 0.02 – 0.35 non-O1/non-O139 *V. cholerae* cases per 100,000 population. Extending surveillance to encompass all human Vibrio infections would help elucidate the burden of disease in the New Zealand population. This would also support New Zealand to prepare for the impacts of climate change on the incidence of vibriosis.

Occurrence of type III secretion system (TTSS) and colix toxin genes in non-O1, non-O139 *Vibrio cholerae* isolated from different sources in Italy

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Non-O1, non-O139 *V. cholerae* can cause gastroenteritis or extraintestinal infections in humans; clinical or environmental isolates can carry virulence factors rather than the cholera toxin. The aim of this study was to investigate the occurence of genes of the TTSS (vcsC and vcsV) and the colix toxin (chxA) in nonO1 nonO139 *V. cholerae* isolated over time from different sources in Italy. Overall, 33 isolates were analysed (19, 5, 1, 6 and 2 from seafood, seawater, freshwater, faeces of diarrhoeal patients and subcutaneous tissue from necrotizing fasciitis, respectively). The ctxA and tcpA genes were absent by PCR in all isolates. Overall, the chxA gene was detected in 42% of the isolates (14 out of 33) of which 33% was from seafood, while none of the isolates from gastroenteritis cases had this gene. TTSS genes were detected in 50% (3 out of 6) of the isolates from gastroenteritis, while the chxA gene was amplified in one of the two isolates from subcutaneous tissues, but the second one did not harbour nor TTSS nor chxA genes. TTSS genes were detected in 11% (2 of 19) and 40% (2 of 5) of the isolates from seafood and seawater, respectively. The presence of virulence genes was variable in the isolates from different sources. However, genes of the TTSS system were detected in half of the isolates from gastroenteritis, while the remaining lacked both TTSS system and colix toxin genes, suggesting that other virulence factors may also play a role in this type of infections.

Determination of provisional epidemiological cut-off values (COWT): a first step to monitor antimicrobial resistance in *Vibrio cholerae* non-O1/non-O139 isolates

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[1]French Agency for Food, Environmental and Occupational Health Safety

V. cholerae is an autochthonous bacterium in aquatic environments such as freshwater, seawater or wastewater. Acquisition of resistance to antimicrobial agents had been frequently described in *V. cholerae* O1/O139, the agent of cholera. In contrast, the literature concerning antimicrobial resistance (AMR) of environmental *V. cholerae* non-O1/non-O139 isolates, is still scarce. However, environmental *V. cholerae* non-O1/non-O139 has been recently proposed as a candidate indicator bacterium to monitor AMR dissemination in aquatic environments. For monitoring purposes, it is of utmost importance to be able to delineate bacterial populations displaying resistant traits, regardless of any

therapeutic outcome. That means being able to categorize microorganisms as wild type or non-wild type, meaning also absence or presence of any acquired resistance mechanism to the drug in question. Epidemiological cut-off values are the most appropriate thresholds to delineate those populations. To date, such values are not available either at Clinical Laboratory Standard Institute (CLSI) or at The European Committee on Antimicrobial Susceptibility Testing (EUCAST). The aim of study was to propose provisional epidemiological cut-off values (CO WT) for *V. cholerae* non-O1/non-O139. The antimicrobial susceptibility of 413 environmental isolates collected between 1999 and 2019 from aquatic environments in three countries (Austria, France, Haïti) was determined. Thirteen antibiotics were tested using the disk diffusion method according to CLSI guidelines. Finally, 11 CO WT were determined using the Normalized resistance interpretation method (NRI). As a next step an increase in the number of participating laboratories is planned to be able to define epidemiological cut-off values for EUCAST or CLSI.

Adaptation of *Vibrio cholerae* to diverse aquatic habitats examined through the observation of Spatio-temporal dynamic of abundances, antimicrobial susceptibility and genomic diversiry

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Spatio-temporal variations of the abundance of culturable *Vibrio cholerae* non-O1/non-O139 and of nine environmental drivers were studied along a salinity gradient under a temperate climate. (France). The four selected sampling sites were representative of three types of water: freshwater, brackish water (2 sites) and marine water. Sampling was performed biweekly in parallel in all sites during 6 months. The peak of *Vibrio cholerae* non-O1/non-O139 abundance was observed in late summer (September-October) in all sampling sites. The highest abundances were recorded at the two brackish water sites together with the highest concentrations of chlorophyll a and pH values. The lowest abundance were observed at both ends of the salinity gradient, freshwater and marine water, respectively. All isolates were susceptible to amoxicillin-clavulanic acid, cefotaxime, meorpenem, chloramphenicol, nalidixic acid, ciprofloxacin, norfloxacin, amikacin, gentamicin, tetracycline, trimethoprim, trimethoprim-sulfamethoxazole and erythromycin. The only resistances were to sulfonamides (86.9%), streptomycin (56.5%), and ampicillin (8.9%). Only nine isolates were multidrug resistant (ampicillin, streptomycin and sulfonamide). No difference was observed between the four sites. Genomic diversity of V. cholerae non-O1/non-O139 was studied by ERIC-PCR. Based on the number of profiles, the diversity was higher in the two brackish water sampling sites than in the freshwater and marine water sites.

Intra-species diversity in geographically distinct *V. cholerae* populations

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Vibrio cholerae is an environmental pathogen that has killed millions of people worldwide in the form of the deadly diarrheal disease cholera in endemic, epidemic and pandemic modes (1, 2). Cholera has been observed to be endemic in many countries, with distinct seasonality in coastal and inland locations (3, 4). Despite significant strides in our understanding of the epidemiology of the disease (2), little is known about the abundance, distribution, and biogeographic structuring of naturally occurring *V. cholerae* populations. In this study, we have analyzed *V. cholerae* populations in distinct geographic zones: cholera endemic Bangladesh (coastal and inland) and cholera free sites on the east coast of USA, to decipher intra-species diversity and population dynamics of *V. cholerae* in diverse ecological settings. We used high throughput amplicon sequencing of a single protein-coding gene, vibriobactin utilization protein subunit B (viuB) that provided subspecies level resolution needed for population analysis. Coastal and inland *V. cholerae* communities were found to be significantly different in terms of diversity and composition. In inland Bangladesh, pandemic generating *V. cholerae* were prevalent throughout the year. Moreover, there was remarkable presence of a novel lineage in noticeably higher abundance in the most densely populated sampling site indicating an anthropogenic influence on the natural *V. cholerae* community. Genomic and phylogenetic analyses suggest that this clade might have divergent ecological roles than typical *V. cholerae* existing in nature. The potential link of human population in their abundance and distribution makes them an intriguing subject for future research targeting Vibrio bacterium adapted to human gut.

Rapid response of *Vibrio* to Green tide-derived dissolved organic matter in seawater

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Vibrio is the fastest-growing marine bacteria and plays important roles in the carbon cycle. In general, *Vibrio* only accounts for a small percentage of total bacteria, but capable to be a dominant group in response to nutrient influx. Although the response of *Vibrio* to poorly characterized factors was studied in special events, which are characterized by suddenness and transience, no direct confirmation of *Vibrio* response to nutrient plus in the laboratory. The microcosm incubation experiments were set up by adding the DOM derived from alga. On the basis of gene-copy numbers and community structure of culturable, active and total Vibrio community, we clarify the whole process of *Vibrio* bloom. As a model for opportunistic bacterial heterotrophs, we demonstrated that Vibrio proliferates in response to DOM additions at rapid timescales. Within 24h of culture, *Vibrio* population bloomed and reached a peak, which accounted for up to 60% of the total bacteria. And *Vibrio parahaemolyticus* was the most active group during the period of *Vibrio* bloom. Meanwhile, the exoenzymes activity in seawater were changed along the fluctuations of *Vibrio* abundance, indicated that *Vibrio* plays a significant role in utilizing DOM. Our study demonstrated the dynamics of *Vibrio* community after DOM influx, shedding light into a promoting effect of the community in the marine carbon cycle.

Temperature and host-adaptation response in the zoonotic pathogen *Vibrio vulnificus*

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Vibrio vulnificus is a worldwide distributed pathogen traditionally isolated from temperate aquatic ecosystems, whose geographical distribution is currently spreading due to global warming. The species is genetically variable and only the strains that belong to the zoonotic clonal-complex (serovar E within pathovar piscis [formerly Biotype 2]) are able to cause disease (most severe form, septicemia) in both humans and fish. Thus, the zoonotic strains differ from the rest of the species in that they can cause septicaemia at 28ºC (warm fish species: main host the eel) and 37ºC (humans). Curiously, the severity of eel disease is strongly dependent on water temperature (non-virulent under 20ºC), while the severity of human disease is strongly dependent on high iron content in blood. The aim of this work has been to unravel the role of environmental temperature on the pathogen host-adaptation response. To this end, we obtained the transcriptome of a representative strain of the zoonotic clonal-complex grown in a minimal medium (CM9) at 20, 25, 28 and 37ºC, and compared them with previously obtained transcriptomes after growing the same strain in eel and human serum as well as in CM9 with and without iron. Transcriptomic results were confirmed phenotypically and by RT-qPCR. Globally, our results suggest that an increase in temperature (from 20ºC to infective temperatures) entail bacterium fitness improvement leading to an optimal physiological state, which in combination with exogenous iron sources, increases the expression of virulence factors in a host-specific manner, and thus contributing to the *V. vulnificus* host-adapted virulent phenotype.

Session 2 – Systematics & Evolution

Establishing a Core Genome Multilocus Sequence Typing Scheme for *Vibrio cholerae* in the Epidemiology of Cholera Outbreaks

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Core genome multilocus sequence typing (cgMLST) has gained popularity in recent years in epidemiological research and subspecies level classification. cgMLST retains the intuitive nature of traditional MLST but offers much greater resolution by utilizing a significantly larger portion of the genome. cgMLST has been successfully implemented in many human pathogens (1–7). Here, we introduce a cgMLST scheme for *Vibrio cholerae*, a bacterium abundant in marine and freshwater environments and the etiologic agent of cholera. We identified 2,443 core genes to analyze a comprehensive dataset of over 1,200 clinical and environmental strains spanning 52 countries. Based on this scheme, we established a sublineage threshold that creates clusters nearly identical to traditional MLST types providing context to new cgMLST classifications. We also defined an outbreak threshold capable of identifying outbreak related strains and potential sources of introduction providing a systematic nomenclature system for epidemiological surveillance. The universality and applicability of

the cgMLST scheme is evaluated by applying it to the two best documented cholera outbreaks in modern history: Haiti and Yemen (8, 9). Advantages of cgMLST is highlighted by a direct comparison with existing classification methods such as MLST (10, 11), multilocus variable number tandem repeats analysis (12), and single nucleotide polymorphism-based method (8). cgMLST outperforms existing methods in terms of resolution, standardizability and usability. We anticipate this scheme will serve as a basis for a universally applicable and standardized classification system for V. cholerae research and surveillance in the future. This cgMLST scheme is made publicly available on PubMLST (https://pubmlst.org/vcholerae/).

Transcriptional response regulator gene arrangements in bacterial species of the Harveyi clade of *Vibrio*

Ana Luisa Vargas Aguilar[1]**,** Beatriz Yáñez-Rivera, Pablo D. Vega-García, Bruno Gomez-Gil

Centro de investigación en alimentación y desarrollo AC

Transcriptional response regulator (TRR) genes represent a significant part of the two-component response regulators of prokaryotes. They function as adaptive switches that couple cellular behaviors to environmental signals through the regulation of gene expression. Species of the Harveyi clade of the bacterial genus *Vibrio*, are a good model to the study these proteins because they are inhabitants of diverse marine environments exposed to fluctuating conditions. This bioinformatic study is focused on the characterization of the TRR gene arrangements in species of the Harveyi clade. The results show that each species has a particular TRR gene arrangement and the association topology patterns of these arrangements is similar to the phylogenetic topology of the clade. The presence of some TRR genes in strains of the same species is variable and this variability could be attributed to gene loss events.

Future considerations for *Vibrio* surveillance in Canada

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[1]Public Health Agency of Canada

The success of enteric disease surveillance in Canada has led to highly detailed knowledge of some of the most important foodborne pathogens. This information spans outbreak response, transmission dynamics, and has influenced national policy. These activities have been limited to a narrow array of high-prevalence bacteria. Due to globalization of our food systems, and impacts of climate change on our costal waters, there is increasing concern for non-traditional enteric pathogens, like *Vibrio spp*. in Canada. The National Enteric Surveillance program (NESP) has been collecting data on the occurrence of *Vibrio* infections in human patients since 1997. Public health labs from across Canada submit case numbers each week for tracking. Traditionally, Canadian *Vibrio*s are speciated, and cholera will undergo serotyping for O1, O139, O75 and O141: the data that are reported to NESP. These data are analyzed in the context of the previous 5+ years of surveillance data to identify trends and possible events. In the last 20 years, there have been 261 *V.cholerae* cases and 175 cases of other *Vibrio* species reported. The most common non-cholerae species that are reported are *parahaemolyticus* (67% of non cholerae strains), *alginolyticus* (13%) and *flubvialis* (10%). This study reports the trends in the *Vibrio* data

collected by NESP over the past 20 years, weaknesses in our surveillance methods and proposes future directions for molecular surveillance of *Vibrio* in Canada. Our goal is to fill the significant gaps in basic knowledge about these organisms that present significant public health risks to Canadians.

Session 3 – Host-microbe Interactions

Title not provided

Marylise Duperthuy

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Biofilm formation is a common strategy used by bacteria in order to survive and persist in the environment. In *Vibrio cholerae*, biofilm-like aggregates are important for the pathogenesis and disease transmission. Biofilm formation is initiated by the attachment of the bacteria to a surface, followed by maturation stages involving the formation of a biofilm matrix. In *V. cholerae*, flagella are essential for the initial step of biofilm formation, allowing the bacteria to swim and to detect a surface. In this study, we explored the effect of polymyxin B, a cationic bacterial antimicrobial peptide, on biofilm formation in pathogenic *V. cholerae* strains belonging to the O1 and O139 serotypes. We found that sub-inhibitory concentration of polymyxin B induces a reduction of the biofilm formation by *V. cholerae* O1 and O139. Experiment on pre-formed biofilm demonstrated that the biofilm formation inhibition occurs at the initial step of biofilm formation, where the flagella are essential. We further characterize the effect of PmB on *V. cholerae* flagellation. Our results demonstrate that the flagellin expression is not reduced in presence of sub-inhibitory concentration of PmB. However, a decrease of the abundance of flagellin associated with the bacterial cells together with an increase in the secretome was observed. Electron microscopy observations also suggest that the abundance of aflagellated bacteria increases upon polymyxin B supplementation. Finally, in agreement with the effect on the flagellation, a reduction of the bacterial motility is observed. Altogether, our results suggest that the polymyxin B affect *V. cholerae* flagella resulting in a decrease of the motility and a compromised ability to form biofilm.

Vibrio cholerae infection induces significant changes in the zebrafish intestinal microbiome

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Zebrafish (Danio rerio) are an attractive model organism for a variety of scientific studies, including host-microbe interactions. Zebrafish contain a core (i.e., consistently detected) intestinal microbiome consisting primarily of Proteobacteria. Furthermore, this core intestinal microbiome is plastic, and can be significantly altered to due external factors. The organism is particularly useful for the study of aquatic microbes that can colonize vertebrate hosts, including *Vibrio cholerae*. As an intestinal pathogen, *V. cholerae* needs to colonize the intestine of an exposed host for any type of pathogenicity to occur. It is suspected that members of the resident intestinal microbial community need to be eliminated by *V. cholerae* in order for colonization, and subsequently disease, to occur. While numerous studies have explored various aspects of the pathogenic effects of *V. cholerae* on zebrafish and other

model organisms, few, if any, have examined how a *V. cholerae* infection alters the resident intestinal community. In this study, 16S rRNA gene sequencing was utilized to investigate how various strains of *V. cholerae* alter the aforementioned microbial profiles following an infection. We found that *V. cholerae* infection and subsequent colonization induced significant changes in the zebrafish intestinal microbiome, with specific members of the microbial community targeted. Additional salient differences to the microbial profile were observed based on the particular strain of *V. cholerae* utilized for challenging the zebrafish hosts. We conclude that *V. cholerae* causes significant modulation to the zebrafish intestinal microbiome in order for infection and subsequent disease to occur.

Investigating *Vibrio parahaemolyticus* ability to combat reactive oxygen stresses from multiple host models of infection

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Vibrio parahaemolyticus serovar O3:K6, the most common cause of bacterial seafood-related illness, is responsible for 48% of all reported *Vibrio sp*. infections in the United States. At this time, limited literature is available regarding organism pathogenesis and especially the host response and immunity to infection. It is well known that pathogenic bacteria may produce enzymes such as catalase (katE1, katE2), alkyl hydroperoxide reductase (aphC1, aphC2) and peroxiredoxins allowing for survival in toxic environments, specifically in the presence of reactive oxygen species (ROS). Toward that end, we have hypothesized that *V. parahaemolyticus* utilizes its antioxidant capabilities, such as katE, aphC, and other antioxidant associated enzymes, to survive in diverse environments, not just in the mammalian host. Here, we investigated the antioxidant genes involved in *V. parahaemolyticus* survival when exposed to toxic oxygen species present in multiple models; in vitro culture, human PBMC infection, murine BMDC infection, and a Galleria mellonella model of infection. We determined that catalases such as katE1 and katE2 but not katG1and katG2 are required for survival in different models of exposure to ROS. Additionally, peroxiredoxins such as glutaredoxin but not prxQ were also differentially required for organism survival in various host environments. At this time, characterization of organism survival in varying environments will allow for a more complete understanding of organism virulence and survival in different hosts and environments providing a comprehensive understanding of *Vibrio parahaemolyticus* pathogenesis. Ultimately, the goal is to formulate treatment algorithms to facilitate pathogenic organism removal regardless of the host or environment.

Title not provided

Leanne Kane

Wellcome Sanger Institute

Within the last ten years, iPSC (induced pluripotent stem cells) have been widely shown to have the ability to be re-programmed to produce a wide range of tissues in the presence of certain growth factors. In this project, we re-direct human stem cells derived from fibroblasts into complex 3D small intestinal structures termed organoids. These organoids have been shown to possess all cell types that are present in small intestinal tissue such as, enterocytes, goblet cells, enteroendocrine cells and Paneth

cells, as well as possessing microvilli and crypt structures. We demonstrate that it is possible to microinject into the lumen of these small intestinal organoids and to manipulate the conditions for infection of non-invasive bacteria such as *Vibrio cholerae* and its toxin. Looking at known bacterial virulence factors, we have shown there are differences in patterns of infection among different strains of *Vibrio cholerae*. In addition, we have shown that the induced human organoids (iHO) elicit a recognisable and measurable host response to bacterial toxin.

Vibrio parahaemolyticus Infection Stimulates Host Cytokine Production and Innate Lympocyte Cell Activation

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Vibrio parahaemolyticus, a gram-negative bacillus, is the main cause of bacterial seafood-related gastroenteritis in the United States. Infection is typically self-limiting; however those with chronic medical conditions or are immunocompromised can be at risk for more severe infections leading to septicemia and death. At this time, in order understand and identify those essential parameters of infection survival we are investigating the host immune response using an intraperitoneal model of systemic infection. First, building upon previously published and established methods, we have documented systemic infection by demonstrating morbidity and dissemination by recoverable organisms isolated from spleen and liver. Additionally, this intraperitoneal infection results in increased IL-6 and TNF-alpha proinflammatory cytokine production, but only marginal IL-1beta production as compared to naïve controls; which all are associated with innate immunity. Interestingly, we determined early after infection, the generation of a CD4+CD69+ effector T cell population and also an increase in a CD8+NK1.1 CD69+ cell population; both of which are being further characterized to fully understand their early role in combating infection to this pathogen. Lastly, at day 30 and day 60 post-infection we were able to detect the production of increased *Vibrio parahaemolyticus* IgG antibody titers. This preliminary data allows us to begin to understand the innate and adaptive response required to eliminate this systemic and often life-threatening infection.

Role of MSHA (Mannose Sensitive Hemagglutinin) pilus in host colonization and biofilm formation in Vibrio vulnificus

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Vibrio vulnificus (Vv) is an aquatic pathogen able to cause septicemia in humans and fish. The species is classified in five phylogenetic lineages and a fish-virulent pathovar named piscis (formerly biotype-2). Pv. piscis is further subdivided in three serovar-related groups, one of which is a zoonotic agent (formerly serovar-E). Regardless the lineage of the strain, the main risk factor predisposing hosts to death by sepsis is a high iron-content in blood. To determine the role of iron in virulence of *Vv*, a transcriptomic study was performed by growing a zoonotic strain (R99) under iron rich and poor conditions. Results showed that several Type IV pili (T4P) and biofilm related genes were putatively regulated by iron. T4P are complex bacterial appendages with critical roles in survival in the environment, attachment and

biofilm production. The aim of this work was to test the role of an iron-regulated T4P, the MSHA (mannose sensitive hemagglutinin) pilus, in fish virulence by comparing a pilus-deficient mutant to the wild-type strain (R99) via in vitro, ex vivo and in vivo assays. Briefly, our results showed that the MSHA pilus is involved in survival of *Vv* in fish serum, virulence by water infection, biofilm formation (carrier fish) and early colonization on eels. In addition, we found a difference in the functions of MSHA pilus between the major *Vv*-lineages. In summary, our results remark the importance of type IV pili in *Vv* survival in and between fish hosts.

Rapid identification of the human virulent *V. vulnificus* strains

Eva Sanjuan

Universidad de Valencia

Vibrio vulnificus is a gram-negative aquatic bacterium found in warm and tropical brackish water that produces infections in humans and fish. This bacterium is found in estuarine and marine environments throughout the world, present in waters, sediments, plankton, molluscs, crustaceans and finfish. This bacterium is a highly invasive pathogen in both humans and fish. Case reports of *V. vulnificus* human infection have been described in several countries throughout the world. The most significant form of *V. vulnificus* disease in humans is a primary septicaemia, which normally follows ingestion of raw or undercooked seafood, mainly oysters and occurs in persons with certain underlying and chronic diseases. Some bacterial factors have been observed to be important for virulence in humans and/or eels; such as the role of the capsule, LPS, or siderophores However, these determinants of virulence have been found in all strains, regardless the origin (clinical or environmental) or the biotype of the strain. Even only a few strains of the diverse population of *V. vulnificus* are associated with disease; there is no test available to measure virulence. Thus, in the absence of definitive information on the contrary, it is assumed that all strains are equally virulent. Here, we present a protocol based in human serum growth that allow the classification of the *V. vulnificus* isolates into three categories that allow the discrimination between the avirulent, the strains that might produces infection in humans with predisposing conditions and the extremely virulent that could produce the infection in all humans.

Optimizing the immunogenicity of a new live-attenuated oral cholera vaccine with serotype engineering

Brandon Sit[1]**,** Bolutife Fakoya, Ting Zhang, Gabriel Billings, Matthew K Waldor

[1]Harvard University

Oral cholera vaccines (OCVs) are a critical component of the global effort to eliminate pandemic cholera. Although currently available OCVs work well in adults, they do not adequately protect young children and require a lag time to stimulate adaptive immunity. We recently developed a new live-attenuated OCV candidate HaitiV, an engineered *Vibrio cholerae* serotype Ogawa strain from the 2010 Haiti cholera epidemic. Pilot studies in two different animal models of *V. cholerae* colonization and disease revealed HaitiV acts not only as a canonical OCV to elicit long-term immunity, but also as a rapid probiotic protective agent against disease in the timespan between vaccination and onset of adaptive immunity.

Here, we describe further optimization of HaitiV to probe the relationship between vaccine serotype and protective adaptive immunity in germfree mice. To explore this question, we constructed a Hikojima serotype variant of HaitiV, which presents both Inaba and Ogawa antigens on its cell surface. Hikojima HaitiV safely colonized adult germfree mice and induced strong anti-Ogawa and Inaba *V. cholerae* vibriocidal antibody responses, similar to Ogawa HaitiV-immunized mice. We are now analyzing the protective capacity of Hikojima HaitiV in these mice with an infant mouse disease model, and performing high-throughput serotyping to investigate the in vivo serotype switching frequency of HaitiV. Data from these studies will provide basic insight into the stability and function of *V. cholerae* serotypes during intestinal colonization and inform the selection of an optimally immunogenic and protective strain of HaitiV for a first-in-human volunteer clinical trial.

Towards a mechanism of action of HaitiV, a probiotic oral cholera vaccine

Bolutife Fakoya[1] and Matthew Waldor

[1]Harvard University

A previous non-lethal exposure to V. cholerae can engender long-term protective immune responses against ensuing *V. cholerae* challenges. Thus, oral cholera vaccines (OCVs) remain important in global attempts to proactively and reactively curb the explosive spread of cholera. We have developed a novel live-attenuated OCV candidate, HaitiV, that provides both in vivo expression of a broad, relevant suite of *V. cholerae* antigens as well as antigen amplification in the small intestine through colonization. In complementary animal models of *V. cholerae* colonization and disease, we have shown that HaitiV engenders long-lasting protection (as a canonical vaccine) as well as provides rapid, probiotic protection against disease with timing inconsistent with adaptive immunity. We have turned to the well-established suckling neonatal mouse model of cholera and demonstrated that this system is indeed equipped to mechanistically characterize this probiotic effect of HaitiV. HaitiV safely colonizes the neonatal mouse small intestine and confers rapid probiotic protection from cholera-like illness. We are currently investigating the underlying mechanism behind this probiotic effect as well as modifications that can be engineered into HaitiV to increase its capacity to confer this rapid protection. Insights from these studies will broaden our understandings of the immediate effects of live-attenuated OCV vaccination, as well as guide the future engineering of HaitiV and its use in human volunteer clinical trials.

Virulence factors Vsm and CopA of the oyster pathogen *Vibrio tasmaniensis* LGP32 confer resistance to marine amoeba predation

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In aquatic environments, free-living amoebae use phagocytosis for nutrition thus exerting a selective pressure on bacterial communities (1). *Vibrio tasmaniensis* LGP32 is an oyster pathogen that behave a facultative intracellular which can resists to the microbicidal activity of oyster hemocytes during phagocytosis (2). Hence, as *vibrios* mostly behave as opportunists we wondered whether LGP32 could

also resist to free-living amoebae found in oyster farming areas. First, as amoebae diversity remains mostly untapped in marine environment, we performed a longitudinal study of marine amoebae diversity during one year in contrasted environments along the French Mediterranean costal waters. Among the sampled amoebae, ones of the Vannella genus were found of particular interest as some of them were found associated with bacteria belonging to the *Vibrionaceae* family and that some Vannella appear to have different susceptibility to different pathogenic vibrios that use different virulence mechanisms to interact with oyster hemocytes (3). By performing functional cell biology, we show that *V. tasmaniensis* LGP32 resist to phagocytosis by Vannella of the Thau lagoon and potentially disturb phagosome maturation processes. Moreover the virulence factor Vsm and the resistance factor CopA were found to be involved in the resistance of LGP32 to predation by Vannella. However some other virulence factors that play a role in oyster infections, do not seem to play a role during the of LGP32 interaction with Vannella. Our work suggests that although some virulence factors might be host-specific, predator-prey interactions between amoebae and Vibrios could select multi-host virulence factors thus promoting opportunism.

Structural biology: Repeats-in-Toxins adhensins are giant biofilm-associated virulence factors secreted by the Type 1 secretion pathway

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Repeats-in-Toxin (RTX) adhesins are a recently discovered class of giant (0.2 -1.5 MDa) biofilm-associated proteins produced by many Gram-negative bacteria including pathogens such as *Vibrio cholerae*, *Salmonella* enterica, and *Pseudomonas aeruginosa*. RTX adhesins are translocated to the cell surface via the Type 1 Secretion Pathway (T1SS), and may initiate biofilm formation by binding bacteria to surfaces and other cells. The extreme size and repetitiveness of these adhesins hampers their characterisation at the molecular level. For example, it is known that V. cholerae uses its RTX adhesin, frhA, to mediate adherence to epithelial cells and help colonize the intestine. However, the specific binding partners of FrhA are unknown due to a lack of detailed structural information. Thus it remains difficult to design specific inhibitors to block RTX adhesins for treatment of infections. Using a dissect-and-build approach, we recently pieced together the first structure of an RTX adhesin called MpIBP, which helps its Antarctic bacterium (Marinomonas primoryensis) form symbiotic biofilms with diatoms on the underside of sea ice. Given that RTX adhesins share the same domain architecture, the structure of the 1.5-MDa MpIBP provides unprecedented insight into the mode of action of these giant virulence factors. The adhesins have an N-terminal domain that retains the adhesin on the cell surface by stopping its passage through the outer-membrane-pore TolC; a long repetitive extender region that projects different ligand-binding domains several hundred nanometers away from the cell surface; and a C-terminal sequence for transporting the protein to the cell outer surface via the T1SS. As proof of concept, structural information about MpIBP was used to model and identify ligand-binding domains of a *V. cholerae* RTX adhesin FrhA, which include those that can bind to various sugar and peptide molecules. Structural studies guided by bioinformatic analyses will identify the domains responsible for helping pathogenic strains form biofilms and cause disease. Our results will also give insight into the rational design of inhibitors that can potentially prevent harmful biofilms and infections.

Characterization of the Zebrafish Immune Response to *Vibrio cholerae*

Dustin Farr[1], Dhrubajyoti Nag[1], Madison Peterson[1], and Jeffrey H. Withey[1]

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Vibrio cholerae is the bacterium that causes the diarrheal disease cholera. Cholera endemics occur largely in developing countries that lack proper infrastructure to treat sewage and provide clean water. One environmental reservoir of *V. cholerae* is fish. Diarrheal symptoms similar to those in humans are seen in zebrafish, a natural host model, as early as 6 hours after exposure. Our understanding of basic zebrafish immunology is currently rudimentary, and no research has been performed exploring the immune response of zebrafish to *V. cholerae* infection. Furthermore, differing strains of *V. cholera* may induce different host immune responses based on that strains virulence factors. We detected a large fold increase in innate cells, inflammatory markers, antibodies, and anti-microbial gene expression during the course of *V. cholerae* infection of zebrafish. Of particular interest was the increase in levels of IL-13, which induced a large increase in levels of mucin secretion that were detected in tank water during infection. Differences in immune responses based on strain were also detected. Our study for the first time characterizes the immune response in zebrafish to Vibrio cholerae infection. These results provide valuable understanding of the natural life cycle of V. cholerae and its relationship with zebrafish, and help in understanding differences and similarities between the immune systems of zebrafish and our own.

"You talkin to me?" Interspecies communication fosters collaboration between closely related symbionts in te sepiolid squid-*Vibrio* mutualism

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The beneficial association between squids in the family Sepiolidae (Mollusca: Cephalopoda) and bioluminescent bacteria in the family *Vibrionaceae* form a unique relationship that provides a model to study the interactions between animals and bacteria. Sepiolid squids from the Mediterranean Sea (genus Sepiola) are unique in that these squids serve as hosts for two bioluminescent bacterial species: *Vibrio logei* and *Vibrio fischeri*. Vibrio bacteria produce unique communication molecules known as acyl-homoserine lactones (AHLs) that are used to modulate light via quorum sensing (QS). Since *V. logei* and *V. fischeri* differ in many of their physiological properties, we examined whether these species produce AHLs that could be "understood" by the other species, and whether the regulatory genes controlling AHL production and subsequently luminescence are genetically distinct. We have identified a number of *V. fischeri* and *V. logei* strains isolated from the same host light organ in order to determine whether both species can "identify" each other's AHLs. Using a biosensor assay, we evaluated the type of AHL that is being produced by each species of *Vibrio*. To determine whether specific luminescence regulators are activated by these AHLs, we created a null mutation on the response regulator gene luxO in *V. fischeri* to determine whether mutations at this locus affect the ability of bacteria to communicate within and between both species during symbiosis. Understanding how different species of bacteria communicate inside an animal host will provide insight as to how symbiotic bacteria evolve cooperative mechanisms in complex beneficial associations.

Human Gut Microbiome Mediates Colonization Resistance to *Vibrio Cholerae*

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My research focuses on interactions between the gut microbiome and the diarrheal pathogen Vibrio cholerae, which causes the severe disease cholera that affects millions of people across the globe. *V. cholerae* must out-compete the gut microbiome to establish itself in the gut and cause disease. We constructed model "susceptible" microbiome and "resistant" human microbiome on the basis of existing metagenomic studies. We then evaluated the effects of these different human gut on *V. cholerae* colonization in an infant murine model of infection. My data showed that healthy microbiomes are resistant to *V. cholerae* invasion. Then to see if there are fluctuations across all healthy individuals' microbiomes, I undertook a study of the range of normal microbiomes in a cohort of 30 healthy adults by collecting fecal specimens and transplanting those microbiomes into antibiotic-treated suckling mice, and assaying for *V. cholerae* colonization resistance. We observed that there is high variability in the ability of healthy microbiomes to respond to *V. cholerae* colonization. Since Blautia obeum's presence played an important role in reducing *V. cholerae* colonization and demonstrated inter-genus differences in Bile Salt Hydrolase (bsh) activity, as well as association with cholera in human populations, we assayed for the level of the B. obeum bsh gene in total DNA extracted from human fecal samples, and found that communities associated with higher *V. cholerae* colonization had lower levels of B. obeum bsh. My future research aims to develop predictive models from this data to identify species strongly able to exclude *V. cholerae*.

Identification of factors that enable enhanced environmental persistence of *Vibrio cholerae* O1 El Tor in the zebrafish natural host model

Madison Peterson[1]**,** Geoffrey B. Severin[2], Christopher R. Rhoades[2], Christopher M. Waters[2], and Jeffrey H. Withey [1]

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Vibrio cholerae (VC) is a gram-negative, aquatic bacterium responsible for causing the human diarrheal disease cholera in millions of individuals living in poverty-stricken regions around the world each year. Over 155 *VC* serogroups exist, but only the O1 and O139 serogroups are capable of causing pandemic outbreaks. The O1 classical biotype is responsible for six global pandemics spanning from 1817 to 1923; however, a new biotype, El Tor, emerged in 1961 to cause the ongoing 7th pandemic. El Tor has since displaced nearly all classical strains as the main source of cholera worldwide and exhibits a unique ability to persist both in the host and external environments for an extended period of time compared to its classical predecessor. Using the zebrafish model, this difference in persistence between biotypes has also been demonstrated to exist in a natural host. Transposon insertion sequencing and complete gene knockouts have been used to identify some of the genes, including colonization factors, critical to El Tor's ability to persist in the zebrafish model. Revealing the mechanisms by which El Tor persists in natural hosts in the environment will enable further understanding of disease transmission and aid in prevention of future outbreaks.

Do *Vibrio* preferentially colonize plastic debris?

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Plastic debris is incredibly abundant in marine environments. Numerous studies have described the microbial colonization of marine plastic debris and some have reported that plastic debris is a vehicle for the transport of potentially pathogenic bacteria (i.e., pathogen hitchhiking). What remains unknown is whether potentially pathogenic species specialize in or preferentially colonize plastic debris. Here, using metagenomic data from our laboratory (N = 12 shotgun metagenomes and N = 161 SSU rRNA metagenomes) as well as publicly-available metagenomics data, we quantified the relative abundance of *Vibrio* species colonizing plastic debris. Data was limited to studies that incorporated a biofilm control in their study design, as taxonomic differences between free-living and substrate-associated lifestyles is a well-documented dichotomy in microbial ecology. We hypothesized that the relative abundance of *Vibrio* would be equivalent on any hard, inert substrate (e.g., plastic, ceramic, and glass). Preliminary results comparing biofilms fouling polyethylene terephthalate (PET), polyhydroxyalkanoates (PHA), and ceramic pellets showed that substrate type did not have a significant effect on Vibrio abundance. Additionally, the relative abundance of free-living *Vibrio* was not significantly different, although it was more variable. These results indicate that *Vibrio* do not preferentially colonize plastic debris. Thus, while *Vibrio* do colonize plastic debris, said debris is not likely to pose a greater risk of pathogen hitchhiking compared to other hard, inert substrates. By contrast, previous studies have shown that organic substrates, like the chitinous exoskeletons of copepods, are indeed preferentially colonized.

Session 4 – *Vibrios* and their Viruses

Prophage encoding double jelly-roll major capsid protein increased biofilm formation of *Vibrio nigripulchritudo*

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Phages that possess double Jelly-Roll (DJR) icosahedral major capsid proteins belong to non-tailed bacteriophages and DJR proteins are found abundant in various ocean viromes. Moreover, prophages that encode DJR capsid proteins are also widespread in diverse bacteria and archaea. However, the functions of these prophages remain poorly understood. Genomic analysis of *Vibrio nigripulchritudo* TKH091, isolated from scleractinian coral Galaxea fascicularis, revealed three prophages encoding DJR capsid proteins, designated Pvn1, Pvn2 and Pvn3. DJR capsid proteins of Pvn2 and Pvn3 are identical but only share 27% identity with Pvn1. Further analysis showed that only Pvn1 encodes complete regulatory structural elements of bacterial DJR viruses such as excisionase, replication A protein and DNA packing ATPase. Pvn1 is integrated at the tRNA-dihydrouridine synthase gene and natural genome excision of Pvn1 was detected during normal growing conditions. Prophage deleted strain ΔPvn1 showed reduced biofilm formation as compared to the wild-type *V. nigripulchritudo* TKH091, suggesting that Pvn1 is

important for the biofilm formation of TKH091. Natural genome excision of Pvn2 and Pvn3 was undetected. Further functional study of Pvn1 and the cross-talk of the three prophages is required to understood the host-phage relationship between bacterial DJR prophages and vibrio host bacteria.

Filamentous phage excision and replication is under the control of the quorum sensing regulator OpaR

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[1]University of Delaware

Vibrio parahaemolyticus is a seafood-borne enteric pathogen found in the marine environment, associated with fish and shellfish. Humans become infected mainly after the consumption of raw or undercooked shellfish. A genetic marker for *V. parahaemolyticus* pandemic strains is the presence of a filamentous prophage f237 (also known as VfO3K6). f237 integrates site specifically into the dif site in chromosome 1 and reproduces via episomal replication. Quorum sensing (QS) is a regulatory mechanism that bacteria use to respond to cell density changes. In a QS response regulator mutant ΔluxO, f237 gene expression was significantly upregulated compared to wild type. In this mutant, the QS master regulator OpaR was constitutively expressed. By performing assays examining for the presence of integrated (attB) and excised forms (attP) of f237, we showed that in the ΔluxO mutant both attB (f237 chromosomal attachment site) and attP (episomal attachment site) levels were increased compared to wild-type. Whereas, in a ΔopaR mutant both attB and attP levels were decreased indicating that OpaR is a positive regulator of excision and possibly f237 replication. To determine whether OpaR directly regulates f237 replication, we examined OpaR binding to the regulatory region of VP1551, an ORF which encodes an RstA homologue involved in replication initiation. Using an electromobility shift assay (EMSA), we show binding of OpaR to the regulatory region. Overall the data suggest that OpaR induces the excision and replication of the f237 prophage.

Session 5 – Genome Biology

Phylogenetic and sequence-based analysis of zonula occludens toxin identified in non-toxigenic *Vibrio parahaemolyticus* strains isolated in Southern Chile

Alequis Pavon

Universidad Autónoma de Chile

Recently, a diversity of prophages encoding zonula occludens toxin (Zot) have been identified in the genome of *Vibrio parahaemolyticus* strains isolated worldwide. To understand the dynamics of Zot acquisition in *V. parahaemolyticus* strains and to evaluate their similarity with functional Zot of *Vibrio cholerae*, *Neisseria meningitidis* and *Campylobacter concisus*, we performed phylogenetic and comparative genome analyses of 53 *V. parahaemolyticus* genomes. Core genome multilocus sequence typing (cgMLST) analyses was congruent with phylogenetic trees of Zot, as strains closely related by cgMLST shared Zot high sequence identity. Sequence and secondary structure comparative analyzes showed that Zot from *V. parahaemolyticus* have the four conserved motifs previously identified in the N-terminal region of *V. cholerae* Zot, but the sequence corresponding to the active fragment (FCIGRL)

located in the C-terminal region is not conserved. Instead, *V. parahaemolyticus* Zot has a conserved secondary structure in the active fragment position compared with other Zots. Finally, a comparison with available 3D protein models showed that the N-terminal, but not the C-terminal, of *V. parahaemolyticus* Zot aligned with the crystallized Neisseria meningitidis Zot. Finally, we have identified that N-terminal region in *V. parahaemolyticus* Zot is highly conserved across other species, more likely due to their crucial role in phage morphogenesis. In addition, despite low sequence identity of the C-terminal of Zot in *V. parahaemolyticus*, we have identified a conserved secondary structure which might have functional consequences in the virulence. We are currently performing experimental strategies to elucidate the role of Zot in the virulence of *V. parahaemolyticus*.

Contribution of the zonula occludens toxin to the virulence of a clinical non-toxigenic strain of *V. parahaemolyticus* and its prevalence on environmental strains from Chilean seafood products

Sebastian Ramirez

Universidad Autónoma de Chile

Vibrio parahaemolyticus is the leading cause of seafood-borne gastroenteritis worldwide. Although TDH, TRH and T3SS2 are present in most of the clinical strains, there are infection produced by strains that lack all these virulence factors (non-toxigenic strains). Prophages encoding zonula occludens toxin (Zot) have been identified in the genome of several strains of *Vibrio parahaemolyticus*. In this work we aimed to i) test the contribution of Zot to the virulence of a clinical non-toxigenic Chilean isolate PMC53.7 and ii) to study the prevalence of the zot gene in Chilean environmental strains of *Vibrio parahaemolyticus* isolated from food matrices. To determine the role of Zot in PMC53.7, we used this purified toxin to test its ability to perturb monolayers of Caco-2 cells. Incubation of cells with a Zot-enriched protein fraction from PMC53.7 altered the structure of the actin microfilaments of cells. Interestingly, the infection with PMC53.7 also modified the Caco-2 cell morphology, clearly affecting the actin cytoskeleton at 3h of infection, in a different way to the pandemic VpKX strain, suggesting that Zot contributes to the virulence of PMC53.7. Additionally, we studied the prevalence of the zot gene in strains isolated from food matrices. Bioinformatics analysis of the zot sequences available for *Vibrio parahaemolyticus* in NCBI, showed seven conserved sites that were used to design “universal primers”. We were able to detect zot in approximately 20% of environmental strains isolated. We are currently studying if Zot could be considered as a novel virulence factor in *Vibrio parahaemolyticus* strains.

The mechanism of Catabolite Activator Protein (CAP) Regulation of *Vibrio* Quorum-sensing Regulated genes

Alyssa Ball[1] and Julia C. van Kessel

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Vibrios use quorum sensing to respond to changes in population density and coordinate group behaviors such as biofilm formation, virulence factor secretion, and bioluminescence. At the center of the quorum-sensing pathway in vibrios is the transcription factor LuxR, which serves as the master regulator to control the expression of >600 genes. Catabolite Activator Protein (CAP) is also a transcription factor

that acts as both an activator and a repressor and has been previously shown to be involved with the expression of bioluminescence in *Vibrio harveyi*. If CAP is deleted, bioluminescence will decrease by 1000-fold. This activation of bioluminescence by CAP was hypothesized to be direct because it was found that CAP can bind to the luxC (bioluminescence) promoter. CAP has been shown to regulate several quorum-sensing regulated genes in *Vibrio vulnificus* and *Vibrio cholerae* as well and there are several instances of LuxR-homolog binding sites near and/or overlapping CAP binding sites; indicating these two proteins could be interacting at promoters in these *Vibrios*. Gel Shift Assays have found 3 possible binding sites in PluxC, two of which overlap with known LuxR binding sites. Protein interaction assays show that CAP and LuxR can directly interact in vitro and the interaction is calculated to be fairly tight. It seems likely that LuxR and CAP interact to activate PluxC but data from competition assays have been found to be contrary to this prediction.

Defining the transcriptional regulatory network driving flagellar motility in *Vibrio campbellii*

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Vibrio campbellii is a gram-negative bacterial pathogen that is both free-living and a pathogen of many marine organisms. Swimming motility is a critical virulence factor in *V. campbellii* pathogenesis, and disruption of the flagellar motor significantly decreases host mortality. However, while *V. campbelli* encodes homologs of flagellar and chemotaxis genes shared by other members of the *Vibrionaceae*, the regulatory network controlling these genes have not been explored in *V. campbellii*. We examined the core flagellar regulators of the flagellar regulatory hierarchy established in other *Vibrios (*rpoN, flaK, flaM, fliA) by constructing knockout mutants of these genes and examining changes in both motility and in the transcription profiles of each mutant. Our data show that while transcription of flagellar and chemotaxis genes occurs via a similar regulatory hierarchy found in other *Vibrios*, the Class II σ54-dependent regulator, FlaK, is dispensible in *V. campbellii*. Additionally, the transcriptome profiles of the regulatory mutants suggest that many Class II flagellar genes in *V. campbellii* may be regulated independently of the established flagellar regulatory hierarchy of other *Vibrios*. Our results establish a new model for regulation of swimming motility in *V. campbellii*, which provides us with new tools to better study motility in *V. campbellii* in the future, as well as to compare flagellar regulation across the *Vibrionaceae.*

Genetic profiling of the presence of potential risk factors in *Vibrio parahaemolyticus (Vp)* isolates from Canadian waters

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Vibrio parahaemolyticus (Vp) is a food pathogen of concern in Canada, impacting the shellfish industry and public health on both Canada's Pacific and Atlantic coasts. The Canadian Food Inspection Agency's (CFIA) coastal laboratories provide significant testing support for the Government of Canada's *Vp* monitoring and control program. Not all strains of *Vp* cause human illness and numerous studies have

shown that the majority of environmental *Vp* isolates lack virulence-associated genes. The CFIA isolates *Vp* from oysters harvested from Canadian coastal waters and analyses them for the presence of two hemolysin genes that are well established to be associated with virulence potential, the thermostable direct hemolysin (tdh) and the TDH-related hemolysin (trh). One hundred and thirteen of these *Vp* isolates were analysed by whole genome sequencing (WGS) in this study to identify virulence markers. Results showed trh was detected in 27 isolates and tdh in only 13 isolates. This low frequency of virulence gene presence amongst *Vp* isolates from oysters harvested in Canadian waters is consistent with findings in other parts of the world. The genome sequences were further assessed for presence of other potential risk factors, including type III secretion systems and markers of antibiotic resistance, to further characterize recent *Vp* isolates from Canadian waters. Warming of global ocean waters may be expected to change the frequency and distribution of pathogenic *Vibrios*, and monitoring of environmental *Vp* isolates for potential risk factors can help identify trends that impact the Canadian shellfish industry.

The transmissible gene qnrVc plays an important and understated role in fluoroquinolone resistance in *Vibrio cholerae*

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The fluoroquinolone ciprofloxacin is a WHO recommended antibiotic for the treatment of severe *Vibrio cholerae* infection. FQ resistance has historically been attributed to topoisomerase (e.g. gyrA, parC) mutations, however the contribution of a FQ resistance gene,qnr*V.cholerae*, remains unclear. Determining the role of qnrVc is important because it is located on an integrating and conjugative element (ICE) and is found broadly across Gram negative taxa. We investigated the genotypic and phenotypic relationship between qnrVc and CIP resistance in *V. cholerae* clinical isolates. Isolates were collected from patients (N=67) during a single cholera outbreak in Dhaka, Bangladesh. Genomes were sequenced and resistance genes were identified using established databases (CARD, ResFinder). The minimum inhibitory concentration (MIC) for CIP was determined by logistic fit of growth (8 μg/ml – 0.00195 μg/ml) and strains were categorized as resistant (MIC ≥ 2 μg/ml) or sensitive (MIC ≤ 1 μg/ml). CIP resistance was found in 54% (36/67). All resistant isolates (36/36) contained qnrVc, which strongly correlated with resistance (McNemar's Chi-squared Test, p = <0.001, kappa = 0.5). The gene qnrVc was the only known FQ resistance conferment in eight isolates with an MIC range of 0.5-2 μg/ml, which is 1-4 fold above what was previously reported. These data suggest that phenotypic CIP resistance can occur via a mechanism independent from topoisomerase point mutations. We advocate that qnrVc be included and prioritized in FQ resistance surveillance.

Genomic insights into multidrug resistance Haitian variant *V. cholerae* circulating in India

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Cholera continues to be a major global concern. Periodic outbreaks are reported from India where cholera is considered endemic. From previous studies, ancestral clone of *V. cholerae* that caused the catastrophic outbreak in Haiti was traced to south Asia. In the present investigation, molecular characterization, toxin production, and resistance profiling of *V. cholerae* strains isolated over a decade (2000-2018) was undertaken and comparative genomics of a representative Haitian variant strain with Classical, El Tor, El Tor variant and Haitian outbreak genomes was done to achieve a profound understanding on its evolution, with emphasis on its virulence and antibiotic resistance determinants. Comparative genomics revealed Haitian variant strains isolated from India to be phylogenetically close to Haitian outbreak strains with respect to cholera toxin operon and core genes. However, T6SS of Haitian variants were identified similar to prototype classical strains. ResFinder and CARD analysis revealed aminoglycoside (strB) and sulfonamide (sul2) resistance genes, with 100% identity, aminoglycoside (strA), phenicol (floR and catB9) and trimethoprim (dfrA1) resistance genes, with 99% identity. Increasing doxycycline resistance in the circulating strains was detected and an adaptive laboratory evolution of the Haitian variant strain was performed to study the effect of doxycycline stress in *V. cholerae*. WGS and SNP profiling of sensitive and laboratory evolved strain revealed mutations in genes encoding drug/ metabolite transporter proteins, 30S ribosomal protein and sensor proteins. The generated data can be utilized for identifying novel therapeutic targets in *V. cholerae*. Also, this study advocates furnishing strict restrictions on the indiscriminate use of antibiotics.

Session 6 – Vibrio Challenges to the Seafood Industry

How depuration processes affects the community of *Vibrio* in Ruditapes philippinarum microbiota

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Vibrio spp. are cosmopolitan bacteria of marine and estuarine ecosystems (1). *Vibrios* are frequently present in seafood especially in filter-feeding mollusks as Ruditapes philippinarum. The Veneto region is one of the main producers of bivalve mollusks where this sea-product has a great economic importance (3). Unfortunately, bivalves are not bacteria-risk-free and the increase of ocean warming is favoring emerging *Vibrio vulnificus* and *V. parahaemolyticus* episodes of infections (2). To ensure a microbiological safe product is necessary to depurate bivalves before their sale to the consumer. Currently, less information are available about the effect of depuration on total community of *Vibrio* present into microbiota of Ruditapes philippinarum. Our project aims to investigate how different depuration facilities and systems affect the quantity and the biodiversity of *Vibrio* species into the microbiota of Manila clam. The study selected four depuration centers in Chioggia (Venezia, Italy), one of these was an experimental closed-system. Aliquots of the same batch were collected before and after depuration in the four depuration centers. Cultural dependent and metabarcoding analysis were applied to the clams' samples. PERMANOVA analysis of *Vibrio* load and microbial counts showed a significant increment on all the post-depuration samples except for the clams depurated in the experimental system. The composition of *Vibrio* community changed with different depuration treatments demonstrating a different response of *Vibrio* community to different depuration processes. In addition, results allowed suitable information to authorities involved in food safety controls and in hygienic management of shellfisheries industry.

Enhancements in rapid quantification of *Vibrio parahaemolyticus*

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Most Probable Number (MPN) using Alkaline Peptone Water (APW) is the preferred method for quantification of *Vibrio parahaemolyticus (Vp*) in oysters. However, this methodology can struggle with other competing *Vibrio* species and can take at least 3 days to provide a confirmed result of viable *Vp*. To improve upon this, we examined the use of an alternative rapid growth broth which we refer to as Yamazaki *Vibrio* Medium (YVM) [1] in combination with a PCR [2] broth screen. Initial testing demonstrated that 1-3 CFU of *Vibrio parahaemolyticus* inoculated into 10 mL of YVM generated levels detectable by PCR or plating within 3-4 hours. However, during a 36 sample validation trial using spiked homogenate cold acclimatized for 72 hours, 4-6% of the 600 YVM-MPN tubes gave false-negative results after 4 hours of incubation. This demonstrates that 4 hours is insufficient for recovery of cold stressed *Vp*. Overnight incubation of the YVM-MPN gave equivalent results to the APW-MPN for both the broth PCR screen and cultural confirmation. Other YVM benefits included: 1) turbidity was more obvious than APW thus only the turbid tubes needed to be pursued; 2) YVM composition and incubation temperature shifted selectivity towards *Vp* which decreased the number of YVM tubes with non-*Vp* induced turbidity. These factors decreased the number of MPN tubes to be pursued by 40% when compared to APW. Based on our results, YVM is an advantageous alternative to APW. Further work is needed to determine the shortest incubation time which will eliminate the YVM-MPN false-negatives.

Inactivating *Vibrio vulnificus* in Pacific Oysters using freezing and frozen storage

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Increasing seawater temperatures both at the surface and in the deep seas are a reality of global climate change. Increases in seawater temperatures will influence bacterial growth and survival. Concentrations of the human pathogen *Vibrio vulnificus* will increase and reach higher numbers in warmer seawater. Oysters are filter feeders and can bio-accumulate pathogenic bacteria from the seawater, thus causing a food safety risk. For the oyster industry, blast freezing and frozen storage have potential as risk mitigation steps. This approach has been previously studied for Vibrio parahaemolyticus but not for *V. vulnificus*. In our study, Pacific oysters (Crassostrea gigas) were put in seawater tanks containing a cocktail of six strains of *V. vulnificus*, to promote bio-accumulation of the bacteria. We then applied blast freezing at –55°C followed by frozen storage at –8°C, –13°C, –18°C, –23°C and –28°C. *V. vulnificus* concentrations were determined at different sampling times from 0-360 days. There was a two-phase inactivation pattern, with rapid inactivation during the first 3 days after freezing, followed by slower log-linear declines during frozen storage. Lower storage temperatures gave slower rates of decline. The effect of storage temperature in *V. vulnificus* numbers (log10 MPN/day) was modelled with regression analysis. From the model, oyster producers/processors can select frozen storage periods at particular temperatures to achieve inactivation of the specific concentrations in their product. This will provide an effective postharvest risk mitigation tool to address the challenge of *V. vulnificus* in oysters.

A 2018 Summer Mortality Event of Pacific Oysters in Baynes Sound, British Columbia is linked to a seawater temperature spike and infection with *Vibrio spp*.

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The Pacific oyster, Crassostrea gigas, is cultured in many regions of the world. In cultivation, adults are prone to mass mortality events during the summer months. A complex combination of environmental and biological parameters has been suggested as the cause of this disease. In recent years, summer mortality has had a significant economic impact on oyster farms in British Columbia, Canada. From late July 2018 to August 2018, up to 87 % mortality was reported in adult Pacific oysters cultivated in Baynes Sound, BC. Farmers in this area first observed mortalities of adult oysters on July 24, which coincided with a spike in sea surface temperatures (SST) in the Sound above the 90th percentile for three days (SST = 20.9oC, 2.7oC above climatology). Histological examination of moribund C. gigas collected during the mortality event revealed early-stage tissue necrosis combined with systemic bacterial infections comprised of bacteria with uniform short-rod-shape morphology. Microbiological characterization – combining 16S rRNA amplicon sequencing, bacterial culture, qPCR, and multilocus sequence typing – revealed the microbiome of moribund oysters were dominated by bacteria related to *Vibrio aestuarianus* and *V. harveyi*. The effect of temperature on *Vibrio* pathogenicity is currently been investigated using laboratory challenge trials, proteomic analysis, and bioassays. Our preliminary findings indicate that increased seawater temperatures alter the abundance, structure, and virulence of *Vibrio* populations associated with the oyster.

Influence of Host Gonadal Stage and Ploidy on Human-Pathogenic *Vibrio* Levels in the Oyster Crassostrea virginica

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While environmental factors strongly influence the distribution and dynamics of *Vibrio vulnificus* and *Vibrio parahaemolyticus*, these factors alone cannot explain why individual oysters in close proximity and experiencing the same environmental conditions present wide variation in abundance of these bacteria. It may be hypothesized that the oyster itself, its health, its physiological condition or its gonadal stage may additionally be important. We designed a study during which hatchery-produced oysters were deployed and analyzed individually for *V. vulnificus* and *V. parahaemolyticus* abundance using a most-probable number approach followed by quantitative PCR, and for gonadal stage as well as other general health factors using histology. Diploid and triploid oysters were co-deployed to examine the influence of ploidy on these human-pathogenic *Vibrio spp*. levels, with sample collection monthly during summer 2018 and 2019. In 2018, *V. vulnificus* and total *V. parahaemolyticus* were present in 100% of the collected oysters. Prevalence of pathogenic *V. parahaemolyticus* strains (tdh and/or trh +) was also very high (> 80%) in early summer and decreased to < 60% in September and October. While prevalence of these bacteria did not appear to be influenced by oyster ploidy, abundance data suggested higher levels of *Vibrio spp*. in diploid compared to triploid oysters during the early summer. The addition of the 2019 data will allow evaluation of whether these observations are repeatable, and

will allow a comprehensive statistical analysis to assess the influence of gonadal stage and ploidy on levels of *V. vulnificus* and *V. parahaemolyticus* abundance in oysters.

Occurrence of *Vibrio parahaemolyticus* in oysters before and after depuration

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The Thau lagoon (French Mediterranean coast), is of economic importance due to bivalve mollusk farming (15,000 t of mussels and oysters a year). The sanitary classification, based on E. coli concentrations in shellfish, of this production area is B. Shellfish must thus be stored in a depuration system for at least 24 hours before sale. However, these sanitary controls do not apply for *vibrios* of human health importance which are present in this lagoon. The aim of this study was to evaluate the presence of *Vibrio parahaemolyticus* in oysters before and after depuration, using MPN-PCR, and to understand the depuration practices of shellfish farmers. Oysters were collected from a production area every 15 days (May-October 2017-2018). Water temperature, salinity and rainfall values were recorded simultaneously. *V. parahaemolyticus* was first detected when water temperature was above 20°C and showed a seasonal dynamic in oysters collected from the production area. The highest concentrations of total *V. parahaemolyticus* were recorded during intense autumn rainfall events (1500 MPN/g). For depurated oysters, *V. parahaemolyticus* was first detected in June 2018 (230 MPN/g). Between July and September (T°C in tanks > 23°C), *V. parahaemolyticus* concentrations (up to 1800 MPN/g) were significantly higher than those in oysters collected in the lagoon at the same period (up to 390 MPN/g). *V. parahaemolyticus* trh2 + was detected in depurated oysters from storage tanks in August 2017 (2.5 MPN/g). These concentrations were significantly correlated with water temperature. An investigation of the stop-sales processes and practices of the professionals in the use and maintenance of the depuration tanks was conducted with a representative sample of 81 shellfish farmers. This survey showed that depuration practices should be improved in order to limit *V. parahaemolyticus* presence in oysters and thus, infections and health issues in humans.

Finding *Vibrio harveyi*: dynamic of bacterial proliferation in fish farming systems of reared seabass

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Vibrio species represent a major concern for food safety. Some *Vibrio* are pathogenic to shellfish and fish, leading to vibriosis. Recently, the Aquanord fish farm in Gravelines (Hauts-de-France), had to deal with a huge decline in sea bass (Dicentrarchus labrax) production, leading to important economic losses. The main cause was vibriosis, caused by Vibrio Harveyi clade species. Despite curative strategies (antibiotics or vaccines), the bacteria persists. Indeed, *Vibrio* has acquired the ability to live in unfavorable environments by forming biofilms on surfaces such as walls of aquaculture ponds. The aim of the present study is to evaluate and understand the *Vibrio* dynamics in the fish farm. We focus on the occurrence of *Vibrio* in 1/ waters of aquaculture ponds, 2/ hot/cold-water inlets and 3/ the occurrence

of Vibrio biofilms on the ponds walls. A 8-months sampling campaign was thus undertaken in order to identify the present *Vibrio* species. Water and biofilm samples were collected in three ponds containing sea bass. More than 1000 bacterial strains were isolated on selective medium (TCBS), and further identified by MALDI-TOF. Moreover, in order to study the *Vibrio harveyi* occurrence dynamics in the fish farm, a qPCR method has been developed to quantify *Vibrio harveyi* in samples, and thus identify the origin of vibriosis development. Preliminary results show a higher abundance of *Vibrio* during warmer months of summer, particularly in hot-water inlet supplying the whole farm. The present study will contribute paving the way towards curing vibriosis in a "clean label" and sustainable development perspective.

Session 7 – *Vibrio* Physiology

rpoB mutations conferring rifampicin-resistance affect growth, stress response and motility in *Vibrio vulnificus*

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Rifampicin-resistance is a worldwide challenge. It is common in different bacterial species and it is typically due to point mutations in the rpoB gene. The rpoB gene encodes for the β-subunit of RNA-polymerase and mutations in this gene cause several secondary effects on fitness, stress response and virulence in different microorganisms [1]. In this work, the secondary effects of rpoB mutations conferring rifampicin-resistance were investigated in the Gram-negative marine pathogen *V. vulnificus*. Three different mutations (Q513K, S522L and H526Y) were identified amongst nine spontaneous rifampicin-resistant strains derivatives of V. vulnificus CMCP6 and different effects on growth, stress response and motility were demonstrated. Both the S522L and the H526Y mutations affect growth in rich medium at 30^{o}C with opposite effects; the former mutation reducing growth rate and overall growth, the latter enhancing growth of *V. vulnificus*. In terms of stress response, the strains carrying the H526Y mutations showed the strongest effect, with a consistent growth reduction in presence of high concentration of NaCl and Sucrose, but, interestingly, not in presence of KCl. Moreover, the same rifampicin-resistant mutants showed a significant reduction in survival in presence of 10% ethanol. Finally, all the rifampicin-resistant strains showed reduced motility, with the greatest effect associated with the H526Y mutation. Rifampicin-resistance has been previously reported in several *V. vulnificus* isolates [2] and is often used for genetic manipulation protocols, with poor consideration of secondary effects of rpoB mutations. Therefore these findings have both clinical and biotechnological relevance.

Multiple modes of cAMP-mediated regulation of the acetate switch in *Vibrio fischeri*

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The gram-negative bioluminescent marine bacterium *Vibrio fischeri* enters into a mutualistic monospecific relationship with the Hawaiian bobtail squid, Euprymna scolopes. To colonize the squid's

light organ, the bacteria must control gene expression to respond to changing nutrient conditions. This involves regulation of the acetate switch, during which *V. fischeri* halts net excretion of acetate and begins assimilating this compound from the environment. The switch requires transcription of acs, which encodes acetyl-CoA synthetase. In E. coli, acs is regulated by cAMP-CRP. While investigating this regulator in *V. fischeri*, we unexpectedly observed that supplementation of growth media with cAMP reduced expression of acs. We hypothesized that cAMP may control acs transcription through a mechanism independent of its role in altering CRP activity. *V. fischeri* can catabolize cAMP using its periplasmic phosphodiesterase, CpdP (Colton et al., 2015; Dunlap and Callahan, 1993; Dunlap et al., 1992), and we confirmed that *V. fischeri* ES114 can grow on cAMP as a sole carbon source. In the cpdP mutant background, we found that acs is no longer repressed in the presence of cAMP, demonstrating that metabolism of cAMP alters acs transcription. We further interrogated the role of CRP and adenylate cyclase in modulation of acs, and suggest that cAMP can act in opposing ways to regulate acs expression levels. Our results are consistent with the conclusion that consumption of a wide variety of nutrient sources, including those molecules that are more typically associated with specific signaling functions, can repress acs in V. fischeri.

CosR is a global regulator of compatible solute biosynthesis and transport in the halophile *Vibrio parahaemolyticus*

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Bacteria accumulate compatible solutes, small organic compounds, in response to osmotic stress to maintain the turgor pressure of the cell. *Vibrio parahaemolyticus* biosynthesizes ectoine (ectABCasp-ect) and glycine betaine (betIBAproXWV) and can transport these compounds or their precursors into the cell using four betaine-carnitine-choline transporters (bcct1 to bcct4) and two ProU (proXWV) transporters. Previously, it was shown that CosR is a negative regulator of ectoine gene expression. In this study, we investigated the role of CosR in glycine betaine biosynthesis and compatible solute transporter gene regulation. Expression analyses demonstrated that the ectoine and glycine betaine biosynthesis genes, bcct1, bcct3, and both proU operons are repressed in low salinity conditions. An in-frame deletion of cosR showed that repression of these gene was mediated by CosR. DNA binding assays demonstrated that CosR binds directly to the regulatory region of each of these genes. In addition, a GFP reporter assay demonstrated that CosR directly represses transcription of betIBAproXWV, bcct3, and proVWX1, and that BetI, a known repressor of its own operon in *V. harveyi*, directly represses transcription of betIBAproXWV. This data shows that CosR is a repressor of the osmotic stress response in low salinity in *V. parahaemolyticus*. CosR repression under low salinity conditions prevents compatible solute biosynthesis and uptake when compatible solutes are not required for fitness.

Molecular and Genetic Investigation of Ascorbate (Vitamin C) Catabolism Amongst the *Vibrionaceae*

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Ascorbate, commonly known as vitamin C, is a ubiquitous 6-carbon carbohydrate characterized by its

ability to scavenge free radicals. In humans and other animals, ascorbate is an essential nutrient involved in enzyme function, as well as tissue repair. Additionally, the ability to utilize ascorbate as a nutrient via fermentation has been described and characterized in various enteric bacteria including Escherichia coli, Klebsiella pneumoniae, and Enterococcus faecalis. In *E. coli*, the Ula system (consisting of ulaR, ulaG, and ulaABCDEF) is required for the fermentation of ascorbate under anaerobic growth conditions. In this study, we identify homologs of the Ula ascorbic acid utilization system within *Vibrio cholerae, Vibrio harveyii, Vibrio campbelli*, and the opportunistic pathogen *Vibrio vulnificus*. We demonstrate that *V. cholerae* and *V. vulnificus* are able to utilize exogenous ascorbate as an energy source supporting growth, while *V. parahaemolyticus*, which lacks the Ula system, is unable to catabolize ascorbic acid. We find that genes corresponding to the Ula system are significantly upregulated in the presence of ascorbic acid as compared to growth in the presence of glucose, or without a major carbohydrate source. Additionally, when grown in minimal media supplemented with mouse intestinal mucus (the primary nutrient source for *V. cholerae* in vivo), ula gene expression is significantly upregulated compared to growth in minimal media supplemented with glucose. We characterize the regulation of these genes in *V. cholerae*. These results suggest that during host-colonization events, vitamin C may be a key carbon source for *V. cholerae*.

Investigation of the quorum sensing regulatory network in *Vibrio parahaemolyticus*

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Bacterial populations alter gene expression in response to changes in cell density via a process called quorum sensing (QS). In *Vibrio* species, the QS response regulator LuxO activates RpoN (sigma factor 54) for transcription of small regulatory RNAs (named Qrrs). Qrr sRNAs activate and repress translation of aphA and luxR mRNA, respectively. We aim to investigate the regulatory components of the QS pathway in *Vibrio parahaemolyticus*, an important human pathogen. In-frame deletion mutants ΔluxO and ΔrpoN were constructed, and surprisingly showed different capsule polysaccharide (CPS) phenotypes, indicating different levels of OpaR (LuxR homologue) in the cell; OpaR has previously been shown to positively regulate CPS production. We found that in a ΔluxO mutant, CPS is produced, however in the ΔrpoN mutant CPS was absent. Quantitative PCR expression analysis of QS genes in ΔluxO and ΔrpoN, relative to wild-type, demonstrated differential expression. In ΔluxO, expression of opaR is significantly upregulated, yet unchanged in ΔrpoN. Expression analysis of the five qrrs showed that qrr2 was significantly upregulated in ΔrpoN, while significantly downregulated in ΔluxO. This suggests that in ΔrpoN, Qrr2 sRNA likely represses translation of OpaR leading to absence of CPS. This was confirmed by examining an ΔrpoN/Δqrr2 double mutant which showed restoration of CPS. We observed that transcription of qrr2 is independent of RpoN in the V. parahaemolyticus QS pathway. Next, we will determine the key regulators involved in qrr2 expression, using a protein pull-down assay, and ultimately elucidate the unique role of Qrr2 sRNA in the quorum sensing pathway.

Carbon, nitrogen and phosphate starvation profile in *V. cholerae* – a case of imperfect nutrient sensing

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Carbon, nitrogen and phosphate starvation profile in *V. cholerae* ¬– a case of imperfect nutrient sensing. In the present study, *Vibrio cholerae* culture was subjected to single, two and three components nutrient limitation (carbon, nitrogen and phosphate). The cells under three and two component starvation enter a suspended survival mode called persister state. However, limitation of single component (carbon, nitrogen or phosphate) was lethal for survival. The limitation of carbon or nitrogen induces stringent response, but with reduced efficiency. The combined effect of carbon and nitrogen starvation induces stronger stringent responses. Interestingly, limiting cells off phosphate eliminated more than 99.99% of population within 48 h of starvation. The presence of carbon and nitrogen appeared to have tricked the cells to assume that nutrients are in plenty. This induces the anabolic activation of reversible rate limiting reactions results in energy spillage (Futile cycle). During phosphate limitation, futile cycling of nutrients increases the production of NADH which can cause membrane damage. Pre-treatment with chloramphenicol to induce stringency had favourable effect of survival under phosphate limitation. We found that, the ability to survive the phosphate starvation varied within the members of Vibrio genus and among other species of gamma-proteobacteria such as E. coli and Salmonella. The CreC two component system, associated with phosphate sensing machinery is absent in *V. cholerae* and appears to be associated with fine tuning the nutrient sensing abilities.

Uncovering the molecular basis of viable but non culturable (VBNC) cells

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Viable but non-culturable (VBNC) cells are cells that are metabolically active, but are unable to form colonies on standard culture media. Following environmental stimuli, such as temperature upshift, some VBNC cells can ‘resuscitate’ restoring their ability to grow on media. Currently, over 80 bacterial species are reported to enter the VBNC state. The ability of VBNC cells to go undetected by conventional microbiological practices could lead to an underestimation of total viable cells in environmental and clinical samples. Furthermore, their capacity to retain virulence potential and their ability for renewed metabolic activity means the VBNC state in pathogens may pose a risk to human health and thus warrants further investigation. Understanding how *V. parahaemolyticus* can reside and adapt when transitioning between environments in order to survive is key to understanding and mitigating against shellfish-associated disease. Furthermore, it is important to understand the risk to human health posed by VBNC cells of *V. parahaemolyticus* in the food chain. This research project has investigated the ability of *V. parahaemolyticus* to form VBNC cells when exposed to stressful conditions. We have developed models to generate *V. parahaemolyticus* VBNC cells in the laboratory and report that different sub populations of VBNC cells can occur based upon their metabolic activity, cell shape and the ability to grow and cause disease in Galleria mellonella. In this study we use mass spectrophotometry to identify and investigate several proteins which play roles in VBNC formation and resuscitation in *V. parahaemolyticus.*

Molecular feedback regulation of σE mediated stress response in *Vibrio cholerae*

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Vibrio cholerae, a Gram-negative highly motile bacterium, is the causative agent of cholera, a severe intestinal human disease, marked by the loss of large volumes of watery stool which can also lead to death. Cholera is contracted by contaminated food or water. In the human gut, *V. cholerae* is assaulted by antimicrobial peptides which act on its membranes [1]. Envelope stress is mediated by the alternative sigma factor σE (RpoE) which eventually activates degP expression encoding for a periplasmic protease/chaperone [2-3]. The outer membrane porin OmpU confers resistance against P2. The exposure of the C-terminal tripeptide motif of OmpU to the periplasm activates the site-1 protease DegS accounting for free σE in the cytoplasm [4]. The key virulence regulator ToxR i.a. inversely regulates the expression of ompT and ompU. In presence of bile salts ompU expression and ompT repression are activated, leading to a resistance to bile salts in the human host [3; 5-6]. Within this study we investigate the molecular cross-talk between σE mediated envelope stress and ompU expression. ΔrpoE deletion strains contain suppressor mutations either in the operator (ToxR binding)- or promoter region of ompU, resulting in altered OmpU levels [7]. Upon insertion of these specific mutations in rpoE+ strains, OmpU levels are reduced, but interestingly they can still elevate OmpU levels in response to bile salts. Using qRT PCR and reporter fusions, we study ompU expression originating either from native or mutated operator/promoter regions in dependence of RpoE and bile salts.

Mechanisms for arsenate tolerance in the pathogen *Vibrio cholerae*

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Vibrio cholerae is a bacterium responsible for the cholera disease. This enteropathogen is a common inhabitant of many marine and freshwater habitats1, where it can be found as a free-living bacteria but also associated with different seafood and fish species2. These animals are known reservoir of arsenic species such as arsenate and other metals. Arsenate can be also present in humans. In fact, arsenate poisons an elevated number of people worldwide through contaminated food and drinking water3. Due to the prevalence of this toxic metal in *V. cholerae´*s hosts, we decided to study the mechanisms for arsenate tolerance in *V. cholerae*. In order to unreveal the determinants implicated in *V. cholerae* tolerance to arsenate, we performed a gobal fitness analysis in the presence of arsenate by Transposon insertion seqeuencing (Tn-seq) and by screening a non-redundant transposon mutant library. We elucidated that *V. cholera* is able to tolerate high concentrations of arsenate mainly due to the coordinated action of a dedicated membrane transporter that expels cytoplasmic arsenate in concert with an unknown phosphatase protein. This tolerance is governed by the LuxO and RpoN regulatory proteins. Our findings explain a novel system in pathogenic bacteria to cope with arsenate toxicity distinct to the canonical detoxification mechanism by reducing arsenate to arsenite. We anticipate that such efficient arsenate detoxification capacity could be exploited by *V. cholerae* to survive and compete with neighburing bacteria in aquatic environments and during host´s colonization.

Session 8 – *Vibrio* Secretion Systems

Gene expression in non-canonical *Vibrio parahaemolyticus* implicates T6SSs in Virulence

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Most *Vibrio parahaemolyticus* isolates found in marine environments are non-pathogenic; however, certain lineages have acquired genomic pathogenicity islands (PAIs) that enable these isolates to cause human illness. The *V. parahaemolyticus* PAI typically contains one or both of two toxins: thermostable direct haemolysin (TDH) or TDH-related haemolysin (TRH) and a type III secretion system 2 (T3SS2). Recently, a few *V. parahaemolyticus* isolates that do not have this PAI were obtained from clinical samples, and there has been interest in determining what the relationship is between these isolates and clinical illness, and whether they possess novel virulence factors. In this investigation, we selected four *V. parahaemolyticus* isolates: a canonical pathogenic strain containing TDH, TRH and T3SS2; two strains from clinical cases which do not contain a known PAI; and an environmental isolate which also does not contain a PAI. For each isolate, we analyzed differential gene expression after crude bile exposure. Several enteric bacterial pathogens, including *V. parahaemolyticus*, are known to use bile as a signal to enhance virulence gene expression. We have shown that in the tdh-positive trh-positive pathotype gene virulence gene expression was not up-regulated in response to crude bile, strongly indicating that the current dogma of virulence gene regulation in *V. parahaemolyticus* needs to be revisited and separately investigated for each pathotype. In addition, we have created a list of genes of interest that were up-regulated in the non-canonical pathotypes which may contribute to virulence in these isolates.