VIBRIO 2014 meeting report

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150 scientists from 26 countries assembled in the John McIntyre Centre at the University of Edinburgh from April 1-4 2014 for VIBRIO2014, a conference dedicated to the study of the biology and biotechnology of vibrios. These Gram-negative aquatic bacteria are widespread in the estuarine and coastal marine environments. They play an important role in marine ecology, e.g. petroleum degradation, and they are also associated with human [e.g. *Vibrio cholerae* the causal agent of cholera] and animal diseases [e.g. *V. anguillarum* the cause of vibriosis in fish). In total, there were 54 oral and 72 poster presentations grouped in 7 sessions. After the official opening by the Scottish Minister for Environment and Climate Change, Paul Wheelhouse MSP, Rita Colwell delivered the keynote address, entitled: "Vibrios past, present and future".

Keynote - Rita Colwell "Vibrios past, present and future."

Dr. Colwell emphasized that cholera is a global disease, being characterized as an acute watery diarrhoea that to date has occurred in more than 50 countries, affecting ~7 million people annually. Cholera has spread extensively since its origin in the Bengal Delta of India, which is regarded as the natural home of the disease. Historically, it has been regarded that there is a relationship between the occurrence of cholera and high temperatures, monsoon conditions and large-scale [human] events leading to poor sanitation.

There have been 7 pandemics to date, with Haiti being the most recently affected country. Research has revealed that outbreaks of disease are often caused by local strains with genetic differences. There were two forms in Haiti, namely epidemic and endemic cholera. The organism is considered to be part of the natural aquatic microflora, with outbreaks of disease linked to rainfall, sea surface temperatures and chlorophyll [= phytoplankton] levels. These influence the zooplankton populations that feed on the phytoplankton. In an attempt to improve monitoring and the accuracy of epidemiological predictions, satellite imagery has been used to measure chlorophyll levels and sea temperature, particularly around Bangladesh, where cholera occurs regularly.

Session 1: *Vibrio cholerae*, Chair: Karl Klose

Vibrio cholerae continues to plague mankind, spreading across the earth in global epidemics, or pandemics, as described by Munirul Alam of the International Center for Diarrheal Diseases Research in Bangladesh.

The Haitian cholera epidemic represents the largest one of the seventh pandemic, with the initial cases occurring at the end of October 2010. The causative strain was identified as *V. cholerae* O1, serotype Ogawa, biotype El Tor[1, 2]. The persistence of cholera in Haiti associated with a seasonal exacerbation of the epidemic during the rainy seasons, especially in coastal areas, prompted Sandrine Baron and Jean Lesne (French Agency of Sanitary Safety) to examine the level of toxigenic *V. cholerae* contamination in the Haitian aquatic environment during the rainy season of 2012. Despite the high levels of *V. cholerae* registered everywhere, they could not demonstrate the presence of toxigenic cells among the samples collected. They concluded that despite its massive dissemination, the toxigenic strain imported into Haiti has failed to colonize the environment to the level required for transmission to humans. This might also explain the lack of gene exchange found between epidemic and environmental strains in Haiti [3, 4].

One of the reasons for the continued success of this pathogen in humans is its ability to acquire new genetic elements through horizontal gene transfer, which drives its evolution. Because *V. cholerae* is also a marine bacterium that lives in aquatic environments during interepidemic periods, its behaviour in the marine environment has a tremendous impact on its ability to cause disease. In the aquatic environment, *V. cholerae* is typically found in close association with the chitin surfaces of plankton and shellfish. Growth on these chitinous surfaces induces *V. cholerae* to take up exogenous DNA and acquire new genetic elements[5]. Melanie Blokesch at the Ecole Polytechnique Fédérale de Lausanne, Switzerland, has elegantly deciphered this chitin-induced competence program, and showed that it is induced by chitin, catabolite repression and cell density-dependent (quorum sensing) signals[6]. With respect to the mechanistic aspects of the DNA uptake process, the Blokesch laboratory has used a cellular biology-

based approach to show that the incoming DNA is most likely pulled into the periplasm of *V. cholerae* through the retraction of a competence pilus concomitant with the binding of the periplasmic DNA-binding protein ComEA.[7, 8] [7]Blokesch has also demonstrated that chitin-induced transformation can facilitate the type of serogroup conversion (acquisition of a new O-antigen gene cluster) that has impacted previous cholera pandemics[9]. Quorum sensing also stimulates the production of the Haemagglutinin protease (HAP), and Malka Halpern at the University of Haifa, Israel showed that *V. cholerae* associated with chironomid (an insect, also known as non-biting midge) eggs uses HAP to degrade these egg masses and control the insect numbers[10-12]. *V. cholerae* HAP production is in turn regulated through inter-species quorum sensing by the complex bacterial community (microbiome) found in the egg masses. Another means of horizontal gene transfer is mediated by Integrating conjugative elements (ICEs) in *V. cholerae* and other *Vibrio* spp. ICEs have facilitated the spread of antibiotic resistance genes among bacteria and thus have a huge impact on human health. Dominic Poulin-Laprade from the laboratory of Vincent Burrus at the Université de Sherbrooke, Canada, described how mobilizable genomic islands can hijack the conjugative machinery of ICEs within the same cell to spread rapidly among bacteria. He also discussed how the transcription factor SetCD regulates this process in response to DNA damage.

To close the session, Karl Klose presented evidence for temperature-mediated virulence gene expression control in *V. cholerae* through the presence of an RNA thermometer located in the 5' untranslated region of the *toxT* mRNA. This strategy, which is novel in *V. cholerae*, controls the translation of *toxT* in a temperature-dependent manner by preventing translation at 20°C and allowing it at 37°C [13]

Session 2 – *Vibrio vulnificus*, Chair: James Oliver

This session began with the keynote talk, "*Vibrio vulnificus* – New Insights Into a Deadly Pathogen", by Craig Baker-Austin from the Centre for Environment, Fisheries and Aquaculture Science. He presented an overview of this most deadly food-borne and wound-causing pathogen, and included the latest information on sequencing of its genome and findings relevant to the three biotypes. The significance of horizontal gene transfer was described, as was the need for better typing methods. An excellent summary of the epidemiology surrounding the disease, including the dramatic gender differences in the victims, as the vast majority of infections occurs in males, was presented. The reports that *V. vulnificus* has recently emerged in a range of temperate regions, such as Northern Europe, clearly emphasize the need for an EU reporting system similar to that of the American Centers for Disease Control and Prevention. Finally, the seasonality of the bacterium, the role of climate in driving the disease incidence, and related risk assessment/prediction was described.

In, his talk "*Vibrio vulnificus*: from NGS [next generation sequencing] to Evolution", Francisco Roig from the University of Valencia presented an update on the latest information regarding the three biotypes, including the amazing number of SNPs present in *V. vulnificus* genomes. Dr. Roig discussed a possible common ancestor and its evolution to the current biotypes, as well as the core genes of this species, and how the two chromosomes differ. However, despite the great advances in genomic analysis of this pathogen, the genes responsible for the virulence of *V. vulnificus* have unfortunately still not been identified.

Tiffany Williams from the University of North Carolina at Charlotte presented an important new finding regarding an aspect of the physiology of this pathogen, namely the viable but non-culturable (VBNC) state. Ms. Williams introduced "Interspecific Quorum Sensing Mediates the Resuscitation of Viable but Nonculturable *Vibrio vulnificus*" with an overview of this dormancy phenomenon and of the involvement of autoinducer 2 (AI-2) in bacterial quorum sensing. She then described how this signal molecule was found to be critical to the resuscitation of dormant cells to the actively metabolizing and culturable state.

These new findings were shown to fit nicely into the "Scout Hypothesis" advanced by Slava Epstein[2]. She ended the presentation by advancing a model for how quorum sensing (the *luxR* AI-2 synthase gene), the stress sigma factor *rpoS*, and catalase all mesh to account for resuscitation of dormant *V. vulnificus*.

Dr. Lien-I Hor from the National Cheng Kung University in Taiwan presented an advance in our understanding of global gene regulation in his talk "Regulation of Virulence by a Homologue of Leucine-responsive Transcriptional Regulator (Lrp) in *Vibrio vulnificus*". She described how her laboratory mapped the mutation responsible for the greatly reduced virulence in mice of a spontaneous mutant in the *lrp* gene, and described several phenotypes resulting from this mutation. These included changes in motility, virulence, cytotoxicity, MARTX toxin levels, colonization potential, and serum sensitivity.

The next talk, "Distribution of Virulence-Associated Traits among *Vibrio vulnificus* Isolates from the Baltic Sea Region" was by Ms. Nadja Bier from the Federal Institute for Risk Assessment in Berlin. Ms. Bier described the incidence of *V. vulnificus* in Baltic waters and the consequences of global warming on its distribution. She described 24 *V. vulnificus* wound infections, and compared the virulence traits of numerous clinical and environmental isolates. Attempts to identify key/differentiating genetic loci between these strains, in connection with their virulence properties, included MLST studies, and examination of serum resistance, mannitol fermentation, *vcg* and 16S RNA sequence, and the presence of sialic acid (*nanA*) and chromosomal region XII.

Following up on the distributional theme, Dr. Brett Froelich from the Institute of Marine Sciences at the University of North Carolina described his research on "Multi-year Changes in *Vibrio vulnificus* Populations in the Neuse River Estuary of North Carolina, USA". Dr. Froelich described how salinity and temperature were the driving forces of these changes. He noted that *V. vulnificus* populations had decreased at the same time as the slowly decreasing level of salinity over several years, in contrast to the expected *V. vulnificus* levels, given the preference of this pathogen for lower salinity. This finding was shown to be due to the

occurrence of an extremely severe drought that affected this estuary during the study period, resulting in a lowering of the *V. vulnificus* population and its replacement by several more highly salt tolerant *Vibrio* spp. This presentation ended with a demonstration of how salinity and temperature could be combined into a predictive "risk map", indicating the times of year when this pathogen occurs in high numbers.

The *V. vulnificus* session ended with a presentation by Prof. James Oliver of the University of North Carolina at Charlotte. His paper, "*Vibrio vulnificus* – Unanswered Questions", challenged the audience to consider, and possibly answer, unanswered questions regarding several unusual aspects of this bacterium and the disease syndromes it causes. These questions included: Why is there a significant age and gender difference in victims? How do the several biotypes and genotypes differ? Why is one biotype unique to Israel and another to eels? Why are human septicaemias, typically resulting from raw seafood consumption, restricted to one genotype? Why are wound infections restricted to a different genotype? Why does one genotype predominate in water and shellfish whereas clinical infections are almost exclusively another? Will global warming result in major increases in geographic distribution of vibrios, their numbers, and in human cases? While these questions were not solved, they led to interesting discussion following the session.

Together, these seven presentations covered a wide range of topics, and summarized much of what we know, and do not know, about this severe human and fish pathogen.

Session 3 – *Vibrio parahaemolyticus*, Chair: Mags Crumlish

This session opened with a keynote presentation entitled "Recent Developments in Seafood Safety with Respect to *Vibrio parahaemolyticus*" delivered by Prof. M. Nishibuchi from Kyoto University in Japan. An excellent overview of the bacterium, clinical incidence and spread of infection throughout the global seafood sector set the scene for the rest of the session[14]. Details of a new pandemic clone identified as 03:K6 was discussed, as was recent evidence regarding the use of *tdh* and *trh* genes as biomarkers for virulence and a rapid and simple method to quantify pathogenic strains of *V. parahaemolyticus* in seafood. Dr. H. Hiyoshi from Osaka University in Japan gave the second presentation in this session, entitled "VOPV, an F-actin-Binding Type III Secretion Effector, is required for *Vibrio Parahaemolyticus*-induced Enterotoxicity". He opened by describing the type III secretion systems (T3SS) of the pathogenic strains where the T3SS2 is required for enterotoxicity expressed by the clinical isolates. His research clearly showed that the novel T3SS2 effector VopV has a specific F-actin-binding activity that is required to stimulate the enterotoxic action of the clinical *V. parahaemolyticus*[15]. In his presentation "Genetic and Phylogenetic Analysis of *Vibrio parahaemolyticus* reveals distinct differences in strains from the Pacific Northwest of the U.S.", Dr. R.N. Paranjpye from the Northwest Fisheries Science Centre, USA presented the results of the use of a range of methods indicating that most of the clinically important strains from the Pacific Northwest are genetically distinct from the isolates associated with the pandemic complex. However, some environmental strains of *V. parahaemolyticus* were found to be similar to the pandemic isolates, in agreement with the biomarker theme provided by the keynote presentation. Dr. Paranjpye summarised that the presence of the *tdh*+ gene was not an adequate predictor of virulence[16]. The next presentation, by Dr. S. Parveen from the University of Maryland, Eastern Shore USA, described the "Incidence of *Vibrio parahaemolyticus* in blue crabs (*Callinectes sapidus*), water and sediments of Maryland Coastal Bays in the United States." The results from a comprehensively performed study showed that the distribution of the pathogenic strains within the sampling sites varied throughout the year. Although *trh*+ isolates were not

240 frequently found during the sampling time, emphasis was placed on ensuring
241 appropriate health and hygiene when handling, cooking and preparing blue crab
242 for consumption to reduce the risk of ill health. Dr. G. Caburlotto from the
243 National Reference Laboratory for Fish, Molluscs, Crustacean Diseases, Padra,
244 Italy, continued the session describing the "Risk of *Vibrio parahaemolyticus*
245 transmission due to local consumption of crustaceans in the northern Adriatic
246 Sea Coasts of Italy." She opened her presentation by explaining that the
247 production of crustaceans is increasing and that through fisheries and
248 aquaculture sources they now represent 8% of world seafood consumption. Her
249 study showed that there was low risk for consumers in the Venice region for
250 transmission of *V. cholerae* and *V. vulnificus* but that genetically heterogeneous *V.*
251 *parahaemolyticus* could be detected at high levels during specific times of the
252 year. Dr. M. Crumlish from the Institute of Aquaculture, Stirling University, UK
253 spoke on "Early Mortality Syndrome (EMS) in shrimp and *Vibrio*
254 *parahaemolyticus*." She provided an overview of the current understanding of
255 the detection methods being applied and the role of this bacterium associated
256 with acute hepatopancreatic necrosis syndrome (AHPNS). This is an emerging
257 infectious disease, causing up to 100% mortality within 30 days in shrimp farm
258 stocks with both white-leg and black tiger shrimp. There is no doubt that this is a
259 novel disease, which is rapidly spreading throughout the shrimp production
260 sector to cause significant production and economic losses[17].

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Session 4 Animal diseases, Chair: Brian Austin

The session focussed on fish and shellfish diseases, and started with a keynote lecture by Prof. Xiao-Hua Zhang from the Ocean University of China, Qingdao, P.R.C., who discussed the problems in Chinese aquaculture, which lost an estimated 1.7 million tonnes of production in 2010 because of disease and pollution. The vibrios, in particular *V. anguillarum* and *V. harveyi,* are regarded as the most important pathogens in Chinese aquaculture. *V. anguillarum* is troublesome in sea bass, flounder and turbot culture, whereas *V. harveyi* causes eye disease and ulceration in these fish. *V. harveyi* is also a major problem in shrimp culture. Quorum sensing affects *V. harveyi* pathogenicity by exerting positive and negative regulation of flagellar motility and haemolysin activity. Interference with quorum sensing, i.e. quorum quenching, was viewed as a possible disease control strategy. Ms. Audrey Vanhove from the Université Montpellier 2, France presented studies on the release of outer membrane vesicles (OMVs) containing a serine protease by *V. splendidus,* a facultative intracellular pathogen of oysters. OMVs were shown to be involved in virulence and resistance to antimicrobial peptides, which contribute to the oyster immune defences, in connection with their involvement in virulence and intracellular survival[18]. The catecholamine stress hormones norepinephrine and dopamine were reported by Tom Defoirdt from Gent University, Belgium to increase the virulence of *V. campbellii* (previously *V. harveyi*) ATCC BAA-1116, a pathogen of crustaceans. They act by enhancing growth in iron-limited environments and by increasing motility and biofilm formation. Subsequent presentations highlighted other oyster pathogens, including *V. tubiashii, V. aestuarianus* and *V. crassostreae,* which are troublesome in French aquaculture. *V. vulnificus* type C was identified by Dr. Jay Grimes of the University of Southern Mississippi, Ocean Springs, USA as the cause of vibriosis that leads to mortality of aqua-cultured red snapper in the USA.

Wax moth larvae were proposed by Stuart McMillan from the Institute of Aquaculture, University of Stirling, UK as a model system or studying the pathogenicity of *V. anguillarum.* The data he obtained in this model are comparable to those obtained in fish challenge experiments. This model system

holds the promise of more rapid progress in understanding and treating or preventing fish diseases caused by this species.

Session 5 - Taxonomy/biodiversity

The round table discussion on taxonomy and biodiversity, organized by Prof. Brian Austin (University of Stirling, UK), Dr. Rita Colwell (University of Maryland, College Park, USA) and Prof. Fabiano Thompson [Federal University of Rio de Janeiro, Brazil), was designed to be thought-provoking, and to stimulate discussion. The value of laboratory cultures and their relevance to fresh cultures was questioned, given the often a rapid loss of biological activity in the laboratory and the consequent differences between cultures from long-term storage and fresh isolates. In particular, the relevance of culturing might be questioned insofar as it is accepted that not all cells grow on laboratory media. Indeed, the culturable proportion of any cell population might be quite limited. This raises the question of how many new and as yet un-described *Vibrio* spp. might be unable to grow on laboratory media. Thus, culture-independent molecular approaches are crucial, although it is important to correct errors in the databases that are used for comparison. However, criticism may be levelled at the current approach of describing new taxa based on the study of only single cultures without evaluating the variability within a species. The study of multiple isolates of any given taxon should be encouraged. Nevertheless, taxonomy has moved forward with the adoption of many modern methods that increase the reliability of the data, and enable sound conclusions to be reached. The fascinating question of the homogeneity of cells within single colonies was also raised in view of the fact that modern approaches allow individual cells to be examined in detail. Indeed, phenotypic variability has been noted in individual cells, leading to the possibility that some genes may only be expressed in certain cells to save energy for the population as a whole.

Phenotypic characterization is mandatory in the description of new taxa. The presentation by Dr. Ana P. Moreira (Federal University of Rio de Janeiro, Brazil), on the new approaches to prokaryotic (vibrio) taxonomy suggested that phenotypes can be predicted directly from genome sequences[19, 20]. According to this speaker, the analysis of genome sequences is enough to define the diagnostic phenotype of new taxa.

Session 6 – Ecology and the environment, Chair: Diane McDougald

The session opened with a keynote talk "Macro-ecology of Vibrios in the temperate northern hemisphere linked to ocean warming" by Dr. Luigi Vezzulli from the University of Genoa, Italy. His talk highlighted the fact that while there is a good understanding of the effect of climate change at the cell and population level, we need to understand better the effects at the community and ecosystem levels. Dr. Vezzulli presented work centred on understanding whether global warming is causing an increase in *Vibrio* numbers and described a novel technique for investigating this question through the use of formalin-fixed samples from the historical Continuous Plankton Recorder (CPR) archive of samples collected from 1948 to 2011. Using 16S rDNA and *gbpA* as a target for PCR, he showed that vibrios, including *V. cholerae,* have increased in prevalence of the last 50 years and that this increase is positively correlated with sea surface temperature.

Ecological interactions of vibrios in the environment was discussed in several talks in this session. Dr. Jay Grimes presented evidence that vibrios degrade petroleum and PAHs in the Northern Gulf of Mexico. Dioxygenases that are required for the degradation of hydrocarbons are possessed by a number of *Vibrio* spp., including *V. cholerae, V. parahaemolyticus* and *V. diazotrophicus*. It was pointed out that this should not be surprising, as vibrios evolved in the oceans where oil seeps are common. Dr. Grimes is investigating 48 vibrio strains for their ability to grow on crude oil, and will analyse the metabolic profile of these cultures after growth to shed light on metabolic pathways that are being used.

Dr Gary Vora from the Naval Research Laboratory, Washington DC, USA reported "A novel *Vibrio* beta-glucosidase and its possible role in the formation of bioluminescent milky seas". Blooms of the photosynthetic alga, *Phaeocystis globosa*, result in large masses of floating colonies that are embedded in a polysaccharide gel matrix. Laminarin, a storage glucan, is produced by these blooms and can support the growth of vibrios. Dr. Vora presented evidence that bioluminescent *V. campbellii* possesses a newly identified gene, *lamN*, which

encodes an enzyme that degrades laminarin to glucose. Strains harbouring this gene were able to utilize laminarin as a sole carbon source and to grow to sufficient cell densities to induce quorum-sensing (QS) phenotypes. Thus, the acquisition of this gene by horizontal gene transfer has enabled this organism to take advantage of deteriorating phytoplankton blooms, and provides a plausible explanation for the possible role of *V. campbellii* in the formation of bioluminescent milky seas.

Dr. Shuyang Sun from the Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore presented the talk "Quorum sensing regulated chitin metabolism provides grazing resistance of *Vibrio cholerae* biofilms". This talk showed that growth of *V. cholerae* on chitin surfaces resulted in the QS-regulated degradation of chitin and production of ammonia. Ammonia was shown to be toxic to heterotrophic protists that feed on bacterial biofilms. Thus, growth on chitin surfaces not only provides a nutrient source but also protects against predation and might provide an explanation for the evolution of QS and the association of vibrios with chitinous surfaces in the environment.

Several talks presented studies on the surveillance of vibrios in the environment and which environmental parameters correlate with their occurrence. Dr. Patrick Monfort from the National Centre for Scientific Research at the Université Montpellier, France presented a talk on the "Dynamics of *Vibrio parahaemolyticus* in the marine system". He investigated the occurrence of pathogenic vibrios (*V. parahaemolyticus, V. vulnificus* and *V. cholerae*) using a most probable number (MPN)-PCR method. His results showed a higher abundance of vibrios in French lagoons compared to seawater, and an increase in the numbers of vibrios following periods of heavy rains that increase flows into the lagoons and thereby decrease the salinity. Salinity, temperature and chlorophyll *a* all have a significant effect on the numbers of *V. parahaemolyticus* in mussels along the French Atlantic coast.

The fact that vibrio infections (wound, ear, eye, GI tract) are increasing in number was discussed in the presentation "Defining the niche of *Vibrio parahaemolyticus* during pre- and post-monsoon seasons in the coastal Arabian Sea" by Prof. Ann-Sofi Rehnstam-Holm from Kristianstad University, Sweden. The abundance of vibrios and algae was determined by real-time PCR. Vibrio numbers were shown to correlate with diatom numbers and chlorophyll *a*. *V. parahaemolyticus* abundance was higher during the pre-monsoon season than the post-monsoon period and was positively correlated with inorganic phosphate and copepod abundance.

Novel methods for detection of vibrios in the environment were presented in several talks. "Quantification of environmental *Vibrio cholerae* with solid-phase cytometry on a large scale" presented by Dr. Alexander Kirschner from the Medical University of Vienna, Austria, discussed the use of a culture-independent method for *V. cholerae* detection. This method uses catalysed reporter deposition fluorescence in situ hybridization (CARD-FISH) in combination with solid phase cytometry for detection and enumeration. Using this very sensitive method, he was able to show a recovery rate of spiked samples of 93-98% and was able to detect between 1 and 550 cells ml^{-1} in the lake and between 4 and 56,300 cells ml^{-1} in shallow saline lakes nearby.

Another novel method presented by Prof Maria Lleo from the University of Verona, Italy was the "Design and validation of a DNA microarray to detect bacterial virulence, antibiotic-resistance and species-specific genes in the marine environment". This technique is highly specificity and highly sensitivity. Using this technique, antibiotic resistance genes were detected in sediment and mussel samples, but sensitivity decreased when contaminants were present. This oligonucleotide-based chip targets a wide range of genes of environmental, economical and clinical interest.

Prof. Carla Pruzzo from the University of Genoa, Italy presented a talk entitled "Aquatic ecology of the oyster pathogens *Vibrio spendidus* and *Vibrio aestuarianus*". By *in vitro* and in field experiments, she observed that *V.*

splendidus maintained culturability and viability for longer than *V. aestuarianus*, the sediment being more suitable for their persistence than seawater. Both vibrios attached to chitin and copepods and formed biofilm but again *V. splendidus* did so more efficiently than *V. aestuarianus.* These findings provide background information on the persistence in coastal water of these bivalve pathogens, thus contributing to a better understanding of the epidemiology of associated diseases.

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Ms Eryn Bernardy from the Georgia Institute of Technology, Atlanta, USA presented a talk on the "Conservation of the regulatory network controlling natural competence in diverse *Vibrio cholerae* isolates". She is working on isolates from Haiti that are not transformable. Despite conservation of the known regulatory components needed for competence (TfoX, HapR and CytR), these isolates were impaired for DNA uptake. All strains were quorum sensing positive and expression of *tfoX* did not restore competence. ChIP-seq and RNA-seq experiments are currently being performed to determine regulatory network for DNA uptake in these isolates.

Finally, Laura Alvarez from Umea University, Sweden presented "Cell wall plasticity within the Vibrionaceae family". She presented evidence that some vibrios have racemases that produce D-amino acids. *V. cholerae* was shown to have D-arginine in the peptidoglycan and high concentrations were also found in the culture supernatant. Further, D-Arg was shown to have a strong lytic activity on a variety of bacteria, indicating that might assist competition against other microorganisms in the environment.

The session ended with a final presentation by Asst. Prof. Diane McDougald from the Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore; "Ecology and the environment, where do we go from here?" This talk highlighted several important concepts for future work. One suggestion was to include aggregate and particle/plankton-associated size fractions for samples obtained for "omic" analyses, as vibrios are often

particle-associated, and due to the way samples are usually collected, are often overlooked as an important member of marine communities. There is also a need to begin to study more complex multi-species or multi-kingdom systems to elucidate interactions. The concept of citizen science was introduced as a viable alternative to ocean sampling, as there are thousands of private vessels that sail the oceans daily. Sampling techniques are easily explained to private citizens and using these resources would increase our coverage of the ocean biosphere. Finally, it is important to begin to integrate the rich ecological knowledge base from eukaryotic biology to microbial systems as an ecological framework. McDougald presented work on the use of eukaryotic chemical defence theory as a framework for the investigation of chemical defences produced by bacteria against protozoan predators.

Session 6 – New Technologies/developments/threats, Chair: Didier Mazel

The keynote presentation in this session, by Prof. Didier Mazel (Institut Pasteur, Paris, France), entitled "NGS approaches of the two chromosomes architecture and maintenance of *Vibrio* genomes" discussed recent work carried out in his lab aimed at understanding the structure of the two *V. cholerae* chromosomes[21, 22]. To this aim, next-generation sequencing-based technologies were used, which include Tn-Seq, optical mapping, and chromosome-conformation capture (3C)-based techniques. These techniques were applied to diverse questions, including understanding the essentiality of Dam in replicating chromosome 2, the observation that *V. cholerae* can survive chromosome 2 replication inactivation through fusing it to chromosome 1, and obtaining a global picture of the inter- and intra-chromosomal structures. The results demonstrate the great potential offered by these next generation sequencing-based technologies in bacterial genetics.

Dr. Carmel Mothersill (McMaster University, Hamilton, Canada) presented her work on the bystander response in fish, entitled "Communication of protective signals from fish sub-lethally challenged with *Vibrio anguillarum* to naïve fish: evidence for a population level survival response?" The bystander response is a phenomenon similar to bacterial quorum sensing in which signals are communicated between stressed cells or whole organisms. Dr. Mothersill's work demonstrated that fish challenged with sub-lethal doses of *Vibrio anguillarum* are capable of conferring resistance to an unexposed partner. These results exhibit characteristics identical to previously established radiation induced signals, and might represent an adaptive response mechanism. This would offer a novel approach for reducing pathogen-induced mortality in fish.

Dr. Tomoo Sawabe (Hokkaido University, Japan) presented work on hydrogen production by *Vibrio* species as a source of energy in a talk entitled "Hydrogen production potential of vibrios: the efficiency, genome structure, and expression." Dr. Sawabe described an evaluation of H_2 productivity of vibrios and

a comparison of the gene structure of the formate hydrogen lysase (FHL) complex within these species. Two groups of vibrios, *V. tritonis* and *V. porteresiae*, demonstrated a high potential for H_2 production[23, 24] [23, 24]. The high level of conservation of the FHL cluster among the vibrios was discussed, though it was noted that differences in gene content within the cluster can change the potential for H_2 production. Culture conditions for carrying out large-scale production of H_2 using *V. tritonis* were also discussed.

Dr. Christine Paillard (Institut Universitaire Européen de la Mer, Université de Bretagne Occidentale, Plouzané, France) gave a presentation entitled, "Paleo-epidemiology in molluscs? A pilot study for identification of pathogenic vibrios in ancient cells." Dr. Paillard discussed a "paleo-epidemiology" approach for understanding bacterial evolutionary genetics, in which bacterial DNA was recovered from shells dating from 1990, representing both healthy shellfish and shellfish with Brown Ring Disease (BRD). This enabled an examination of the shellfish microbiomes. Such an approach would allow assessment of the evolutionary history of mollusc populations and microbial communities from the European "Little Ice Age", which would enable analysis of these factors during eras of climate change. Dr. Paillard concluded her talk by requesting that anyone with available samples send them to her for study.

Dr. René Erler presented her work in a talk entitled "Clear Vibrio species identification with MALDI-TOF MS Vibriobase". She described whole-cell matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a technique that provides a rapid and cost-effective means of identifying bacterial species, and discussed the creation of VibrioBase, a collection of 1076 vibrio strains which provides alignment scores more specific for identifying vibrio species as compared to the Bruker database more commonly used in MALDI-TOF identification. MALDI-TOF is a powerful tool for species identification, as it can be used for subspecies identification along with

MLSA, and can be used to analyse mixed cultures. VibrioBase will provide a powerful tool for species surveillance.

Dr. Neelam Taneja's talk, "Surveillance of cholera in endemic inland regions having fresh water environments in India" discussed the re-emergence of cholera in India in the 2000's. This work highlights the incidence of cholera outbreaks in inland areas, with a focus on outbreaks in Chandigarh, where *V. cholerae* emerged in 2002. Environmental site and patient monitoring found that strain N16961 was present only in cholera-affected areas, while the presence of non-O1/nonO139 strains was dependent on environmental factors. Genome comparisons revealed that environmental strains form closely related groups, and *V. cholerae* outbreaks were shown to result from "rapid expansion" of a population at a particular time. One of the conclusions of this presentation was that O1 strains were not isolated from fresh water, and were only recovered from cholera-affected areas. A model was proposed that would monitor areas at risk for favourable cholera outbreak conditions by measuring parameters such as air and water temperatures, salinity and pH, and take appropriate action to reduce the risk of cholera transmission in these areas when an outbreak is likely.

Dr. Jyl Matson (University of Toledo, Toledo, USA) presented a talk entitled, "Analysis of the *Vibrio cholerae* stress response using RNAseq", which described bacterial responses to antimicrobial peptides encountered in the environment. A Mariner transposon screen was performed on *V. cholerae* El Tor to find genes associated with resistance to the antimicrobial peptide polymyxin B. Genes mapped in this screen included MsbB, AlmE (which possesses a frame-shift mutation in the Classical strain), and VC1639, a sensor histidine kinase which is part of a two-component system along with VC1638, the two-component system itself being similar to PhoQ. An RNASeq assay on *V. cholerae* strain C6706 and on a VC1639 mutant either with or without polymyxin B treatment yielded an overlap of up-and-down-regulated genes in the presence of polymyxin B. One gene that was up-regulated in C6706 was VCA0732, encoding a hypothetical

protein similar to YgiW, which is an OB-fold protein. Mutants of YgiW are somewhat sensitive to polymyxin B, however, mutants of VCA0732 were not sensitive to polymyxin B treatment. This work provides an excellent example of the power of RNAseq to understand gene regulation under various environmental conditions, which is made easier because of the small bacterial genome.

A talk by Dr. Alfonso Soler (Institut Pasteur, Paris, France), "*S10-spec-α* repositioning provides insight into genome organization rules of fast-growers" discussed the importance of chromosomal gene location for bacterial growth. In this work, the *S10-spec-α* locus was relocated to various regions throughout the *V. cholerae* genome. This highly conserved 13.2 kb gene locus contains half of the ribosomal protein genes, as well as the α subunit of RNA polymerase, and is located close to the origin of replication in fast-growing bacteria. When this locus was moved to regions on chromosome 1 35, 510, and 1120 kb away from its original location near the origin of replication, or to the replication termination (ter) region of chromosome 2, the growth rate declined in proportion to the distance from the original location. The strains possessing the relocated locus also demonstrated reduced ability to infect *Dropsophila melanogaster*. This work provides the first experimental evidence of the gene dosage hypothesis accounting for genome organization in fast growing bacteria, and demonstrates the power of "recombineering" approaches for answering biological questions.

The following presentation by Anna Newton (Centers for Disease Control and Prevention, Atlanta, USA) was entitled, "Vibriosis transmission in the United States, 1988 – 2012". This presentation outlined the vibrio monitoring system used by the Centers for Disease Control and Prevention (CDC) for tracking vibrio-related diseases throughout the USA, called Cholera and Other *Vibrio* Illness Surveillance (COVIS), and described the manner in which vibriosis cases are reported to the CDC. Her talk gave an overview of the percentage of vibrio species responsible for vibriosis cases reported in the US, and described the

differences that could be observed in these cases in terms of region and seasonality. The incidence of vibriosis in the US has doubled from 1988 to 2012. Limitations of the reporting system were also discussed. Recommendations for reducing the instances of vibriosis included taking preventative steps towards reducing the likelihood of vibrio transmission through food, and the use of prevention messaging to increase public awareness of infection risk.

The final talk of the session was given by Dr. Felipe Cava (MIMS-Molecular Infection Medicine Sweden at Umeå University, Umeå. Sweden), "The MUREINome: deciphering cell wall plasticity in bacteria towards the development of a new generation of specific antimicrobials". After presenting an overview of the bacterial cell wall, and its role in morphology and protection against environmental threats, Dr. Cava discussed how non-canonical D-amino acids (NCDAA) are released by diverse bacteria and are incorporated into the cell wall in a highly promiscuous manner. The team has designed a suite of NCDAA labelled with differently coloured fluorophores that allows them to follow cell wall growth and morphogenesis. The differences in cell wall modelling between bacterial species enables an encyclopedic annotation of cell walls, which would allow the classification of bacterial species based on cell wall composition and structure, what the authors term an "e-murein-barcode", which could also be used to investigate cell wall traits in environmental species such as viable-but-non-culturable bacteria. Dr. Cava outlined a project to generate a comprehensive database of peptidoglycan diversity and plasticity, termed the MUREINome. The authors used Ultra Performance Liquid Chromatography-Mass Spectrometry to analyse peptidoglycan within the Vibrionaceae family, and discovered a large diversity in cell wall composition, finding over 30 different components. Their search for novel peptidoglycan units could assist the search for new antibiotic targets.

Conclusions

The *Vibrio* radiation includes more than a hundred species, which show a conserved and specific genome structure coupled with a high content variability reflecting the extent of horizontal gene transfer in the aquatic environment. Although the majority of the presentations were centred on species that are pathogenic for human or animals, from their ecology down to the characterization of specific macromolecular complexes and pathways, the conference also highlighted the wealth of studies on the biology of other *Vibrio* species and their potential uses in biotechnology. The attendees agreed to the need for a further conference, and Roscoff in France, which was proposed by F. Le Roux for hosting VIBRIO2016 from March 29th to April 1st, 2016, was chosen.

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