

VIBRIO 2014 meeting report

Didier Mazel, Rita Colwell, Karl Klose, James Oliver, Mags Crumlish, Diane Mcdougald, Michael Bland, Brian Austin

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1 **VIBRIO 2014 Meeting Report.**

2

3 Didier Mazel¹, Rita Colwell², Karl Klose³, James Oliver⁴, Mags Crumlish⁵, Diane
4 McDougald⁶, Michael J. Bland¹, and Brian Austin⁵

5

6 ¹, Unité Plasticité du Génome bactérien and CNRS UMR 3525, Département de
7 Génomes et Génétique, Institut Pasteur, Paris, France.

8 ², Maryland Pathogen Research Institute and Center of Bioinformatics and
9 Computational Biology, University of Maryland, College Park, MD 20742, USA.

10 ³, Department of Biology, University of Texas San Antonio, San Antonio, TX 78249,
11 USA.

12 ⁴, Department of Biology, University North Carolina at Charlotte, Charlotte, NC
13 28223, USA.

14 ⁵, Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK.

15 ⁶, Centre for Marine Bio-Innovation, School of Biotechnology and Biomolecular
16 Science, The University of New South Wales, Sydney 2052, Australia.

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18 Corresponding author : Didier Mazel, mazel@pasteur.fr

19

20 150 scientists from 26 countries assembled in the John McIntyre Centre at the
21 University of Edinburgh from April 1-4 2014 for VIBRIO2014, a conference
22 dedicated to the study of the biology and biotechnology of vibrios. These Gram-
23 negative aquatic bacteria are widespread in the estuarine and coastal marine
24 environments. They play an important role in marine ecology, e.g. petroleum
25 degradation, and they are also associated with human [e.g. *Vibrio cholerae* the
26 causal agent of cholera] and animal diseases [e.g. *V. anguillarum* the cause of
27 vibriosis in fish]. In total, there were 54 oral and 72 poster presentations
28 grouped in 7 sessions. After the official opening by the Scottish Minister for
29 Environment and Climate Change, Paul Wheelhouse MSP, Rita Colwell delivered
30 the keynote address, entitled: "Vibrios past, present and future".

31

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

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

40

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

51

52

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

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

57

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

72

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

86 based approach to show that the incoming DNA is most likely pulled into the
87 periplasm of *V. cholerae* through the retraction of a competence pilus
88 concomitant with the binding of the periplasmic DNA-binding protein ComEA.[7,
89 8] [7]Blokesch has also demonstrated that chitin-induced transformation can
90 facilitate the type of serogroup conversion (acquisition of a new O-antigen gene
91 cluster) that has impacted previous cholera pandemics[9]. Quorum sensing also
92 stimulates the production of the Haemagglutinin protease (HAP), and Malka
93 Halpern at the University of Haifa, Israel showed that *V. cholerae* associated with
94 chironomid (an insect, also known as non-biting midge) eggs uses HAP to
95 degrade these egg masses and control the insect numbers[10-12]. *V. cholerae*
96 HAP production is in turn regulated through inter-species quorum sensing by the
97 complex bacterial community (microbiome) found in the egg masses. Another
98 means of horizontal gene transfer is mediated by Integrating conjugative
99 elements (ICEs) in *V. cholerae* and other *Vibrio* spp. ICEs have facilitated the
100 spread of antibiotic resistance genes among bacteria and thus have a huge
101 impact on human health. Dominic Poulin-Laprade from the laboratory of Vincent
102 Burrus at the Université de Sherbrooke, Canada, described how mobilizable
103 genomic islands can hijack the conjugative machinery of ICEs within the same
104 cell to spread rapidly among bacteria. He also discussed how the transcription
105 factor SetCD regulates this process in response to DNA damage.

106

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

113

114

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

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

130

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

139

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

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

152

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

161

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

172

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

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

187

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

202

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

206

207

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

209 This session opened with a keynote presentation entitled “Recent Developments
210 in Seafood Safety with Respect to *Vibrio parahaemolyticus*” delivered by Prof. M.
211 Nishibuchi from Kyoto University in Japan. An excellent overview of the
212 bacterium, clinical incidence and spread of infection throughout the global
213 seafood sector set the scene for the rest of the session[14]. Details of a new
214 pandemic clone identified as 03:K6 was discussed, as was recent evidence
215 regarding the use of *tdh* and *trh* genes as biomarkers for virulence and a rapid
216 and simple method to quantify pathogenic strains of *V. parahaemolyticus* in
217 seafood. Dr. H. Hiyoshi from Osaka University in Japan gave the second
218 presentation in this session, entitled “VOPV, an F-actin-Binding Type III Secretion
219 Effector, is required for *Vibrio Parahaemolyticus*-induced Enterotoxicity”. He
220 opened by describing the type III secretion systems (T3SS) of the pathogenic
221 strains where the T3SS2 is required for enterotoxicity expressed by the clinical
222 isolates. His research clearly showed that the novel T3SS2 effector VopV has a
223 specific F-actin-binding activity that is required to stimulate the enterotoxic
224 action of the clinical *V. parahaemolyticus*[15]. In his presentation “Genetic and
225 Phylogenetic Analysis of *Vibrio parahaemolyticus* reveals distinct differences in
226 strains from the Pacific Northwest of the U.S.”, Dr. R.N. Paranjpye from the
227 Northwest Fisheries Science Centre, USA presented the results of the use of a
228 range of methods indicating that most of the clinically important strains from the
229 Pacific Northwest are genetically distinct from the isolates associated with the
230 pandemic complex. However, some environmental strains of *V. parahaemolyticus*
231 were found to be similar to the pandemic isolates, in agreement with the
232 biomarker theme provided by the keynote presentation. Dr. Paranjpye
233 summarised that the presence of the *tdh*+ gene was not an adequate predictor of
234 virulence[16]. The next presentation, by Dr. S. Parveen from the University of
235 Maryland, Eastern Shore USA, described the “Incidence of *Vibrio*
236 *parahaemolyticus* in blue crabs (*Callinectes sapidus*), water and sediments of
237 Maryland Coastal Bays in the United States.” The results from a comprehensively
238 performed study showed that the distribution of the pathogenic strains within
239 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].

261

262

263 **Session 4 Animal diseases, Chair: Brian Austin**

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

291

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

296 holds the promise of more rapid progress in understanding and treating or
297 preventing fish diseases caused by this species.
298
299

300 **Session 5 - Taxonomy/biodiversity**

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

325

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

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

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

346

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

357

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

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

372

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

383

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

396

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

406

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

417

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

426

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

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

437 .

438

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

448

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

456

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

463 particle-associated, and due to the way samples are usually collected, are often
464 overlooked as an important member of marine communities. There is also a need
465 to begin to study more complex multi-species or multi-kingdom systems to
466 elucidate interactions. The concept of citizen science was introduced as a viable
467 alternative to ocean sampling, as there are thousands of private vessels that sail
468 the oceans daily. Sampling techniques are easily explained to private citizens and
469 using these resources would increase our coverage of the ocean biosphere.
470 Finally, it is important to begin to integrate the rich ecological knowledge base
471 from eukaryotic biology to microbial systems as an ecological framework.
472 McDougald presented work on the use of eukaryotic chemical defence theory as
473 a framework for the investigation of chemical defences produced by bacteria
474 against protozoan predators.
475

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

477 The keynote presentation in this session, by Prof. Didier Mazel (Institut Pasteur,
478 Paris, France), entitled “NGS approaches of the two chromosomes architecture
479 and maintenance of *Vibrio* genomes” discussed recent work carried out in his lab
480 aimed at understanding the structure of the two *V. cholerae* chromosomes[21,
481 22]. To this aim, next-generation sequencing-based technologies were used,
482 which include Tn-Seq, optical mapping, and chromosome-conformation capture
483 (3C)-based techniques. These techniques were applied to diverse questions,
484 including understanding the essentiality of Dam in replicating chromosome 2,
485 the observation that *V. cholerae* can survive chromosome 2 replication
486 inactivation through fusing it to chromosome 1, and obtaining a global picture of
487 the inter- and intra-chromosomal structures. The results demonstrate the great
488 potential offered by these next generation sequencing-based technologies in
489 bacterial genetics.

490

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

502

503 Dr. Tomoo Sawabe (Hokkaido University, Japan) presented work on hydrogen
504 production by *Vibrio* species as a source of energy in a talk entitled “Hydrogen
505 production potential of vibrios: the efficiency, genome structure, and
506 expression.” Dr. Sawabe described an evaluation of H₂ productivity of vibrios and

507 a comparison of the gene structure of the formate hydrogen lysase (FHL)
508 complex within these species. Two groups of vibrios, *V. tritonis* and *V.*
509 *porteresiae*, demonstrated a high potential for H₂ production[23, 24] [23, 24].
510 The high level of conservation of the FHL cluster among the vibrios was
511 discussed, though it was noted that differences in gene content within the cluster
512 can change the potential for H₂ production. Culture conditions for carrying out
513 large-scale production of H₂ using *V. tritonis* were also discussed.

514

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

527

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

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

539

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

555

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

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

574

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

590

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

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

605

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

627

628 Conclusions

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

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