Vibrio2017: the Seventh International Conference on the Biology of Vibrios

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ABSTRACT  Vibrio2017, the ASM Conference on the Biology of Vibrios, was held in November 2017. The conference focused on all aspects of biology related to the bacterial genus Vibrio. The meeting highlighted that the vibrios have a tremendous impact on humans, both directly in the form of Vibrio-related diseases and indirectly through their interactions with other animal species, e.g., fish and shellfish, and with our environment, including influencing the health of our coastal waters and coral reefs.

KEYWORDS  Vibrio, Vibrio cholerae, Vibrio fischeri, Vibrio parahaemolyticus, Vibrio vulnificus, biofilms, cholera, environmental microbiology, immunization, secretion systems

Gram-negative bacteria within the Vibrio genus have a huge impact on the biology of the earth. A number of Vibrio spp. (particularly Vibrio cholerae, Vibrio parahaemolyticus, and Vibrio vulnificus) cause significant human disease. Indeed, the Centers for Disease Control and Prevention estimates that there are 80,000 cases of Vibrio infections in humans in the United States every year (1), and V. cholerae alone is thought to infect as many as 1.4 to 4 million persons globally each year (2). Vibrio spp. also cause disease in marine vertebrates and invertebrate animals, including fish, shrimps, and corals. These interactions have a significant impact on ecology, such as destruction of coral reefs, but also on economies, particularly through the impact on aquaculture. Yet vibrios can also establish symbiotic relationships with hosts, such as Vibrio fischeri with squid, serving as a model for understanding of beneficial host-microbe interactions. Vibrios also have set the paradigm for understanding of general bacterial physiological processes, such as quorum sensing, flagellar assembly, multiple chromosome segregation, biofilm formation, type VI secretion, and virulence gene regulation. They are also a model organism for natural transformation and horizontal gene transfer and for genetic evolution, especially during disease epidemics. Vibrios are also becoming a bellwether organism for studies of the influence of climate change on water-related diseases.

The Vibrio2017 conference took place from 12 to 15 November 2017 in Chicago, IL, an appropriate location given the history of cholera epidemics there in 1849, 1854, and 1866. Vibrio2017 continued a successful series of international conferences focused on Vibrio spp., previously held in Ghent, Belgium (2005), Paris, France (2007), Rio de Janeiro, Brazil (2009), Santiago de Compostela, Spain (2011), Edinburgh, Scotland (2014), and Brest, France (2016). There were 174 participants from 22 different countries in attendance. The goal of the meeting was to highlight common themes among the diverse fields of Vibrio research, focusing on physiology, environmental persistence,
interactions with aquatic animals, interactions with humans, and omics/regulation. Some of the notable commonalities are discussed below.

**VIBRIO MODULATION OF HOST/COMPETITORS**

*Vibrio* spp. are well known to express and secrete toxins that modulate eukaryotic host cell behavior, most notably, the cholera toxin of *Vibrio cholerae* that is associated with the severe, life-threatening diarrhea caused by cholera. Since the discovery of cholera toxin and the *ctx* genes, extensive efforts have been made to create strains of *V. cholerae* that do not produce this toxin, in order to create safe vaccines, with several now in clinical use (3). Matt Waldor presented his group's latest efforts to modify a *V. cholerae* strain isolated from Haiti as a live attenuated vaccine. In animal studies, this strain showed protection against *V. cholerae* colonization and mortality as rapidly as 1 day after exposure, indicating that this potential vaccine could be used as a probiotic to immediately prevent disease spread during epidemics. Ed Ryan and colleagues also discussed their recent data on how live attenuated cholera vaccines can drive long-term protection through development of antibodies against the O-polysaccharides of *V. cholerae*.

Recently, it has been appreciated that vibrios can also employ delivery of toxic effectors to neighboring bacteria to shape the surrounding bacterial community, most prominently via the type VI secretion system (T6SS) that was discovered by John Mekalanos, Stefan Pukatzki, and colleagues (4). The T6SS is a contractile sheath that delivers effector molecules into bacterial or eukaryotic cells (Fig. 1). John Mekalanos gave the keynote address for the conference, highlighting how *V. cholerae* uses T6SS to kill competing bacteria to gain a competitive advantage for successfully colonizing the intestine. He also discussed how intestinal precolonization with T6SS-active (nonpathogenic) bacteria could be developed as a type of probiotic, but for use in aquaculture, to prevent colonization by pathogenic *V. parahaemolyticus*, which is transmitted through contaminated seafood. In another link to our understanding of T6SS, Stefan Pukatzki and colleagues discovered through genomic sequencing that *V. cholerae* strains carry different effectors, dictating whether they compete or coexist with their neighboring bacteria. Because T6S is coupled to natural transformation, this provides vibrios with a means to acquire new effectors when they kill neighboring bacteria and to take up their genomic DNA, thus allowing them to diversify and compete in new niches.

Brian Hammer showed that *V. cholerae* not only utilizes the T6SS to compete against other bacteria within the zebrafish intestine but also injects an actin cross-linking effector into intestinal cells to modulate the intestine itself through altered peristalsis, presumably facilitating its own persistence within the intestine. Type III secretion systems (T3SS) are another device used by a number of *Vibrio* spp. to inject a variety of effectors into host cells, through a needle-like syringe device. Kim Orth described the
two different virulence-associated T3SSs of *V. parahaemolyticus*. One particular effector, VopL, inhibits the host reactive oxygen species (ROS) response that would otherwise kill the bacteria, thus promoting their intracellular survival. Karla Satchell discussed yet another means that vibrios use to modulate the host. The multifunctional autoprocessing repeats-in-toxin (MARTX) toxins carry an array of effector domains that modulate the host cell to promote pathogen virulence. She detailed how an effector domain of the *V. vulnificus* toxin is linked to increased virulence by cleaving the host small GTPase Ras. Carmen Amaro presented a description of how these toxins can cause a cytokine storm within the host that accounts for the rapid lethality associated with *V. vulnificus* infections in humans. Adèle James reported also the discovery of a new MARTX toxin that is associated with particularly severe disease in oysters.

Yet another mechanism that vibrios use to secrete various components is the mechanism involving the outer membrane vesicles (OMVs), which are small round blebs from the outer membrane that carry molecules within them. Edward Ruby described OMVs secreted by *V. fischeri* that emanate from their sheathed flagella. The sheath that envelops the *Vibrio* flagellum is composed of outer membrane material, but its synthesis and function remain a mystery. *V. fischeri* forms a complex symbiotic relationship with the bobtail squid by colonizing its light organ and producing light. Ruby showed that the OMVs produced by *V. fischeri* contain the lipopolysaccharide (LPS) and peptidoglycan that are used to trigger the development of the squid host light organ. Andy Camilli discussed how the OMVs produced by *V. cholerae* have another function, namely, to act as decoys in defense against bacteriophages (Fig. 2). Bacteriophages bind to the surface of their prey in order to inject their genetic material.
and ultimately kill the bacteria. *V. cholerae*-specific bacteriophages bind to the OMVs and nonproductively inject their DNA into them instead of into the bacteria, a phenomenon described in detail in this issue of the *Journal of Bacteriology* (5). Brameyer et al. demonstrate yet another function of OMVs in *V. harveyi* in this issue, describing how quorum-sensing autoinducer molecules are trafficked between cells via OMVs (6).

**VIBRIO STRUCTURES**

Due to genetic tractability, the vibrios are among the bacterial organisms that have contributed greatly to our understanding of basic bacterial physiology and structural biology. In this conference, the impact of new technologies in driving our understanding of large protein complexes was evident. Grant Jensen discussed how his laboratory utilized electron cryotomography to uncover the elegant structural details of the T6SS device (Fig. 1). Further, he presented new models of the *V. cholerae* toxin-coregulated pili that are essential for intestinal colonization and virulence. By coupling tomography with bacterial genetics, he was able to demonstrate how loss or addition of large tags on different structural components can facilitate our understanding of how these nanomachines are assembled and function. Lisa Craig then presented new data on how the *V. cholerae* TcpB protein contributes to toxin-coregulated pilus retraction.

The powerful impact of electron tomography on increasing our understanding of bacterial surface structures was also highlighted by Michio Homma. He presented a three-dimensional reconstruction of the flagellar basal body derived by electron cryotomography. He then discussed how vibrios are able to uniquely place a single flagellum located at the bacterial pole. Karl Klose and colleagues further presented information describing how the flagellar filament is comprised of five different flagellin subunits but only one specific flagellin, FlaA, is necessary and sufficient for filament formation; the reason for this is described within this issue (7).

**VIBRIO PERSISTENCE IN THE ENVIRONMENT**

The aquatic environment serves as the reservoir of vibrios operating to continuously reintroduce pathogens into the human food supply and as a source for novel niche adaptation driving the evolution of new pathogenic variants. Vibrios persist in the environment because they form biofilms, which are resistant to environmental stresses, and they interact with other aquatic lifeforms and/or enter into nonculturable/persister modes of slower metabolism.

Vibrio long-term persistence in the aquatic environment is associated with the ability of the bacteria to form biofilms (bacterial communities encased within a matrix attached to solid surfaces) (Fig. 3). Over the past decade, the laboratory of Fitnat Yildiz has been studying the developmental process that *V. cholerae* uses to form biofilms. Her research has revealed a number of insights into this process, including the central role that the internal signaling molecule cyclic di-GMP plays in regulating biofilm formation (8). While the role of the extracellular *Vibrio* polysaccharide (VPS) in forming the biofilm matrix surrounding the bacteria has been studied extensively, the presence of proteins within the matrix and their function in biofilm formation have been somewhat mysterious. Yildiz showed that the RbmA matrix protein binds to the polysaccharide and undergoes a binary structural switch that alters VPS-dependent higher-order structures, demonstrating that the matrix proteins also play an important role in biofilm architecture. Amanda Lewis discussed how vibrios produce vertebrate-type sialic acid-like sugars as part of the LPS to serve as a type of “cloaking device” that disguises the bacterium from the host, ultimately facilitating virulence.

Vibrios are ubiquitous in the marine environment but usually make up a minor portion of the microbes there. However, vibrios are known to bloom under conducive environmental conditions, and these population increases are correlated with increased disease incidence in host organisms, including humans. Erin Lipp discussed how the prevalence of vibrios in the marine environment is limited by a lack of iron. Saharan dust storms deposit minerals, including iron, into the marine environment, and vibrios are then able to utilize the increased iron levels to rapidly proliferate, leading Lipp to
propose that the iron within Saharan dust is a major driver of these blooms, which dramatically alter the marine bacterial composition to be dominated by vibrios within just 1 to 2 days. Martin Polz presented how an ensuing bloom of vibriophages can then drive the crash of vibrios, occurring almost as rapidly as they appear.

Climate change may also be contributing to these blooms not only through increasing the size of deserts and Saharan sandstorms but also by expanding the geographical distribution of *Vibrio* infections (9, 10). It is predicted that rising water temperatures will increase the risk of exposure to vibrios, resulting in increased illness (11). In fact, *Vibrio* infection rates have tripled in the United States over the course of a 15-year time span (1996 to 2010) (12). Fabiano Thompson described how increased water temperature can also lead to increased prevalence of certain bacterial species, for example, *V. coralliilyticus*, within coral reef ecosystems, leading to dysbiosis and coral disease, such as white plague disease. Orr Shapiro used microfluidics and nanoscale secondary ion mass spectrometry to study the initial interactions of *V. coralliilyticus* with coral polyps, which revealed the oral epidermis to be a likely point of entry for the bacteria (Fig. 4). The oral epidermis of coral is lined by motile cilia whose beating likely concentrates the bacteria in this location, in strikingly similarity to the manner in which the cilia of the bobtail squid concentrate *V. fischeri* to colonize the light organ as mentioned above (13).

**GENOMICS AND EVOLUTION**

The impact of the dramatic influx of data through the use of whole-genome sequencing was evident across all sessions of the meeting, revealing the genomic flexibility of the *Vibrio* genus. This has led to a greater appreciation of the role of horizontal gene transfer (HGT) in the evolution of vibrios. One mechanism utilized by these bacteria for HGT is the induction of natural competence, which occurs when they grow in the presence of chitin (14). Chitin is ubiquitous in the marine environment, and vibrios can be found in close association with various chitinous surfaces found on zooplankton, shellfish, etc. Growth on chitin induces the expression of the regulatory protein TfoX, which in turn activates the expression of the T6SS and the competence machinery. Activation of T6SS leads to killing of competing bacteria (as detailed above) and release of their genomic DNA, which is then taken up by the competence machinery. Through use of high-resolution fluorescence microscopy, Ankur Dalia was able to directly visualize the process of DNA uptake during horizontal gene transfer by
natural transformation in *V. cholerae*, which involves a type IV pilus that captures DNA outside the cell and threads it across the outer membrane. Artificial induction of TfoX expression bypasses the need for chitin for transformation, and this can be exploited as a tool for genetic manipulation in vibrios (15); Orth and colleagues describe utilizing this technique for the genetic manipulation of *V. parahaemolyticus* in this issue (16).

The high rates of HGT that take place in the natural environment of vibrios contribute to the evolution of these bacteria but are not the sole drivers. Whole-genome sequencing of *Vibrio* strains has allowed a clearer picture of the accompanying genetic changes, e.g., speciation, and an investigation of speciation within the Harveyi clade by Henryk Urbanczyk and colleagues, described in this issue (17), indicated that gene gain and/or loss did not contribute to speciation but rather to stochastic diversification of local populations. Yan Boucher described tracking the evolution of pandemic *V. cholerae* strains by whole-genome sequencing, which revealed that specific ecological interactions within the marine environment shape seasonal cholera epidemics, because they influence the abundance and distribution of specific *V. cholerae* strains.

**CONCLUDING REMARKS**

*Vibrio*2017 succeeded in bringing together scientists from all over the world who work on this amazing group of marine bacteria. Many of the presentations discussed using cutting-edge microscopy and genetic and high-throughput technologies to reveal novel aspects of *Vibrio* biology. Further, the meeting highlighted common aspects of bacterium-host interactions, environmental persistence, and genome evolution. Toronto, Canada, was selected as the site of the next international *Vibrio* meeting: *Vibrio*2019. See you there!

**REFERENCES**


