The Lifestyle of Vibrio cholerae Fosters Gene Transfers

Growing on chitinous surfaces helps these bacteria to initiate horizontal gene transfer and, perhaps, to swap pathogenic traits.

Melanie Blokesch

Waterborne Vibrio cholerae bacteria cause cholera, a pandemic during the last 50 years across Asia, Africa, and Latin America. Although most infected individuals do not develop severe symptoms, some become violently ill with severe diarrhea, vomiting, and cramps, and the loss of body fluids, if untreated, can lead to shock and death.

V. cholerae, which is primarily encountered in estuaries, rivers, and coastal waters in its environmental reservoir, is found not only in a free-living state but also associated with phytoplankton and zooplankton (Fig. 1). Because factors such as increasing temperatures, the El Niño phenomenon, and heavy rainfalls and floods can raise the abundance of phytoplankton and zooplankton, and, along with them, cholera bacteria, changing climatic conditions could well drive the re-emergence of cholera, according to Rita Colwell from the University of Maryland and her collaborators.

Moreover, V. cholerae’s strong association with the chitinous exoskeletons of zooplankton, which serves as a source of nutrition but also contributes to the onset of natural competence in these bacteria, could mean that chitin surfaces are hotspots for transformation that foster the evolution of V. cholerae and other vibrios. In a broader sense, by learning more about the environmental lifestyle of V. cholerae, we can better understand the mechanisms by which this seemingly innocuous microbe becomes a harmful human pathogen of great importance. This knowledge, in turn, may help us in predicting the onset of cholera outbreaks and facilitate efforts to control them.

V. cholerae in the Environment

The epicenter of past cholera pandemics is the delta area where the Ganges and Brahmaputra Rivers flow into the Bay of Bengal. In this region and elsewhere, V. cholerae is often found attached to small crustaceans and their molted exoskeletons, and it emerges periodically as a human pathogen. Such exoskeletons are made primarily of chitin, a homopolymer of branched chains of β-1,4-linked N-acetyl-D-glucosamine (GlcNAc).

During the last decade, researchers realized that the link between this microbe and chitin surfaces is of relevance to public health. As V. cholerae forms biofilms on such chitin surfaces, the bacteria concentrate, reaching levels of up to $10^4$ to $10^6$ bacteria on single copepods (a dominant member of the zooplankton). Ingesting a few of those small crustaceans can be sufficient to cause disease in humans. Notably, chitin also increases the bacterium’s resistance to acid, meaning fewer bacteria are killed while passing the stomach, thus increasing the chances of establishing infections. Hence, drinking a single glass of contaminated water during a zooplankton bloom in cholera-endemic regions can lead to the onset of disease.

Knowledge of the environmental lifestyle of this pathogen and how it is transmitted to hu-
Humans can help in decreasing cholera cases. For example, Rita Colwell and her collaborators introduced a simple filtration method, using folded sari cloth to remove particles larger than 20 μm, including zooplankton, from drinking water. Trial use of this method during 3 years in 65 Bangladeshi villages with a total population of 133,000 reduced cholera cases by 48% compared to a control group.

**V. cholerae Associates with Chitinous Surfaces**

Marine zooplankton produce more than $10^{11}$ tons of chitin annually, providing a huge substrate on which *V. cholerae* can attach and form biofilms, protecting them against external stresses. *V. cholerae* not only binds to chitinous exoskeletons; it also plays an important role in remineralizing chitin, preventing much of this material from ever reaching the ocean floor even though chitin is the most abundant polymer in marine environments—equal to cellulose on land. The recycling of chitin by *V. cholerae* and other bacteria helps to maintain the global carbon and nitrogen cycle, according to the late Saul Roseman of Johns Hopkins University in Baltimore, Md., who pioneered research on chitin degradation by the *Vibrionaceae*.

Colonization of chitinous surfaces by *V. cholerae* is a consequence of (i) sensing and chemotaxis; (ii) attachment and biofilm formation; and can lead to (iii) chitin-induced natural competence, leading to DNA uptake and transformation; and (iv) evolution, if new genes are acquired (Fig. 2). The bacteria can secrete chitinases that degrade chitin throughout all these steps.

There are two classes of chitinases: exochitinases, which release diacetylchitobiose [GlcNAc]$_2$ units from the nonreducing end of the polysaccharide chain, and endochitinases, which produce multimers of GlcNAc by randomly cleaving the glycosidic bonds within the chitin polymer, eventually releasing free oligosaccharides.
Once attached to the chitin surface by a type IV pilus (mannose-sensitive hemagglutinin pilus) and the GlcNAc-binding protein GbpA, the bacteria form microcolonies that develop into biofilms, according to Ronald Taylor and colleagues at Dartmouth College in Hanover, N.H. Maturation of biofilms involves both protein components and exopolysaccharides, both of which help to form the matrix that holds bacterial cells within these biofilms, according to Fitnat Yildiz at the University of California, Santa Cruz, and Paula Watnick at Boston Children’s Hospital, and their collaborators. Biofilm-forming V. cholerae attach to chitinous crab shell fragments via assorted fibers and filaments, according to our scanning electron micrographs (Fig. 3).

**Horizontal Gene Transfers among V. cholerae in Aquatic Environments**

How did V. cholerae gain pathogenic traits in the first place? The genome sequence of V. cholerae and genomic hybridization experiments suggest horizontal gene transfers. For example, the cholera toxin phage CTXφ resides as a prophage in the V. cholerae genome and is transferred to new strains via phage transduction, according to Matthew Waldor and John Mekalanos at Harvard Medical School in Cambridge, Mass. In addition, its two pathogenicity islands as well as the Vibrio seventh pandemic islands, the gene cluster encoding the O-antigen region of the lipopolysaccharide (LPS), an integrative and conjugative element, and other regions of the two chromosomes bear hallmarks of recent gene acquisitions.

Several mechanisms enable bacteria such as V. cholerae to obtain DNA from external sources. For example, chitin induces natural competence for genetic transformation in V. cholerae, according to work done by Gary Schoolnik and his collaborators at Stanford University in Stanford, Calif., in which I also participated. Natural competence, a mode of HGT, refers to the physiological state in which a bacterium can take up free DNA from the environment and incorporate that material into its own chromosome by homologous recombination. If the transforming DNA material carries a different O-antigen gene cluster (shown in green as part of the transforming DNA), V. cholerae can exchange this region, thereby undergoing serogroup conversion (e.g., change of the O-antigen, illustrated on the right).
V. cholerae Associates with and Responds to Chitin under Laboratory Conditions

The chitin polymer’s insolubility complicates efforts to study how V. cholerae associates with it. As one approach, we use Dungeness crab shells, which we clean, break into small pieces, and sterilize by autoclaving. V. cholerae readily colonize such surfaces when they are submerged in an artificial sea water medium within 12-well plates (Fig. 4). We also use commercially available coarse chitin flakes aliquoted into tubes to better standardize experimental procedure and to lower the risk of cross-contamination. Moreover, to visualize bacterial attachment to chitin surfaces, we use chitin beads, which are amenable to light microscopy (Fig. 4).

Although V. cholerae encompasses more than 200 different serogroups specified by the O-antigen region of the LPS, only strains belonging to the O1 serogroup were thought to cause severe cholera. In the 1990s, a V. cholerae that converted from the El Tor O1 serogroup to the new O139 Bengal serogroup caused cholera outbreaks in Bangladesh and India. This unprecedented event was followed by spread of the O139 serogroup strain to other countries and fear of a new eighth cholera pandemic.

To test whether V. cholerae O1 can acquire the O139 gene cluster by chitin-induced natural transformation under laboratory conditions, we propagated a V. cholerae O1 El Tor strain as a biofilm on crab shell fragments and then added...
genomic DNA from the O139 Bengal strain as transforming material. We then selected for O1-to-O139 transformants by using a virulent bacteriophage specific for the O1 serogroup to carry out serogroup-specific, phage-mediated killing. The resulting phage-resistant transformants were genotypically identical to the O1 recipient except for the acquired O139 serogroup gene cluster (42 kbp) and the lost O1-specific gene cluster (32 kbp). Testing with immunological and electron microscopy-based methods showed that the serogroup convertants produce the new O-antigen.

These results provide a mechanistic explanation for the appearance of the O139 Bengal variant of *V. cholerae* in 1992. More generally, they illustrate how genetic and ecological factors can lead to the emergence of new pathogenic microbes. Indeed, because the O-antigen clusters of different serogroups of *V. cholerae* display a GC-content that deviates from the rest of the chromosome, chitin-induced natural transformation could well be responsible for the diversity of natural *V. cholerae* serogroups. Moreover, because serogroup changes in toxigenic *V. cholerae* strains, as observed for the O139 serogroup, might reoccur at any time, diagnostic tools should take this scenario into consideration.

**Regulatory Network Drives Chitin-Induced Transformation in *V. cholerae***

Our recent research focuses on the regulatory network that drives natural competence and transformation in *V. cholerae*. Although *V. cholerae* cells respond uniformly to soluble chitin oli-
gomers, competence induction around insoluble biotic chitinous surfaces is nonsynchronized and heterogeneous. Because the chitin surface can serve as the sole carbon source for *V. cholerae*, we investigated the link between carbon catabolite repression and chitin-induced natural transformation. Indeed, both colonization of the chitin surface and induction of natural competence depend on the secondary messenger cyclic adenosine monophosphate (cAMP) in *V. cholerae*. Moreover, quorum sensing (QS) also contributes to the onset of natural transformation in *V. cholerae*.

Through QS, bacteria synthesize autoinducer (AI) molecules and secrete them into the environment to measure and respond to population density. Briefly, *V. cholerae* depends on two QS circuits, according to Bonnie Bassler at Princeton University and her collaborators. The system 1 cholera autoinducer 1 (CAI-1) serves as the signal for intraspecies communication. System 2 is used for interspecies communication, which depends on autoinducer 2 (AI-2), a furanosyl borate diester.

How do these environmental signals foster natural competence and transformation? First, we learned that QS regulates only a small subset of competence genes and that this regulation occurs via a transcription factor that links chitin-induction and QS. More precisely, QS mediates a switch from DNA degradation around *V. cholerae* cells towards competence-mediated DNA uptake. Further, chitin-induced natural transformation depends on the species-specific signal, CAI-1, whereas the interspecies autoinducer AI-2 plays a negligible role. Importantly, when both autoinducers are absent, natural transformation is abolished.

These results illustrate how *V. cholerae* can enhance species-specific DNA uptake, namely...
by coupling gene expression required for natural transformation to the species-specific QS system. This linkage could prove helpful in repairing damaged genes. To follow up on this assumption we are now also studying the mechanistic aspects of the DNA uptake process in *V. cholerae*.

Melanie Blokesch is an Assistant Professor at the Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland, melanie.blokesch@epfl.ch

**Acknowledgments**

I thank Graham Knott (BioEM facility of EPFL) for his help and enthusiasm with the electron microscopy. I also acknowledge all current and former members of the Blokesch lab for their scientific contributions. Finally, I thank Gary Schoolnik at Stanford University for introducing me into this fascinating topic. Research in the Blokesch laboratory is supported by grants from the Swiss National Science Foundation (31003A_127029 and 31003A_143356) and by a Starting Grant from the European Research Council (VIR4ENV).

**Suggested Reading**


