Viruses in corals: hidden drivers of coral bleaching and disease?

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Marine viruses are the largest, but most poorly explored genetic reservoir on the planet. They occur ubiquitously in the ocean at an average density of 5–15 × 10^6 viruses per mL of seawater, which represents abundances an order of magnitude higher than those of bacteria. While viruses are known agents of a number of diseases in the marine environment, little is known about their beneficial function to corals. Herein, we briefly introduce the topic of viruses as potential drivers of coral bleaching and disease.

Increasing prevalence of coral bleaching and disease

Corals form a symbiosis with microscopic algae (*Symbiodinium* spp.), which are the primary carbon source of their host through translocation of photosynthates. The loss of these intracellular symbionts is referred to as coral bleaching, causing the coral tissue to pale and resulting in a vulnerable state of the coral animal[1].

In recent years, coral bleaching and diseases have increasingly contributed to coral mortality for a number of reasons. First, warm seawater temperature anomalies that lead to mass bleaching events have increased in frequency and have left corals less time to recover[2]. Such temperature anomalies have been associated with higher disease incidence, possibly due to increased activity of pathogenic bacteria at elevated temperatures combined with reduced immunocompetence of stressed corals[3]. Second, the growing spatial scale of anthropogenic impacts on coral reefs such as reduced water quality[4] and tourism activities[5] have also been linked to higher disease prevalence. For example, up to 15-fold higher coral disease prevalence was reported on reefs in the Great Barrier Reef that had tourist platforms compared to those without[5]. Third, the frequency and severity of cyclones and crown-of-thorns starfish predation have increased; these disturbances cause breakages and injuries to corals and provide entry points for pathogenic microorganisms[6,7]. Despite the increase of coral disease occurrence, the tools required for rapid diagnostics are still lacking and management strategies to prevent and mitigate coral disease outbreaks are largely inadequate[8]. Of prime concern is that causative agents have not been identified for the majority of the described coral diseases. While a few known scleractinian coral pathogens are bacteria[9], the role of viruses in coral health and disease has barely been examined.

Virus diversity in corals

Coral-associated virus communities are highly diverse and comprise bacteriophages, archaeal and eukaryotic viruses[10–12]. Despite this diversity, only a smaller subset of taxonomic groups are commonly found in corals, including bacteriophages belonging to the order of the *Caudovirales*, and eukaryotic nucleocytoplasmic large DNA viruses (NCLDVs) belonging to the families *Phycodnaviridae*, *Mimiviridae*, *Poxviridae* and *Iridoviridae*, as well as *Polydnavridae* and *Retroviridae*[10–12]. The coral-associated viral diversity shows that viruses could infect all cellular members of the coral holobiont, i.e. the coral animal, algal symbionts and all of its other microscopic and macroscopic symbionts.

Eukaryotic viruses in coral disease and bleaching

Although over 20 coral diseases have been described, none of them are unequivocally shown to be caused by a eukaryotic virus that directly infects the coral animal or symbiotic algae (Figure 1A). For example, yellow band/blotch disease (YBD) causes degradation

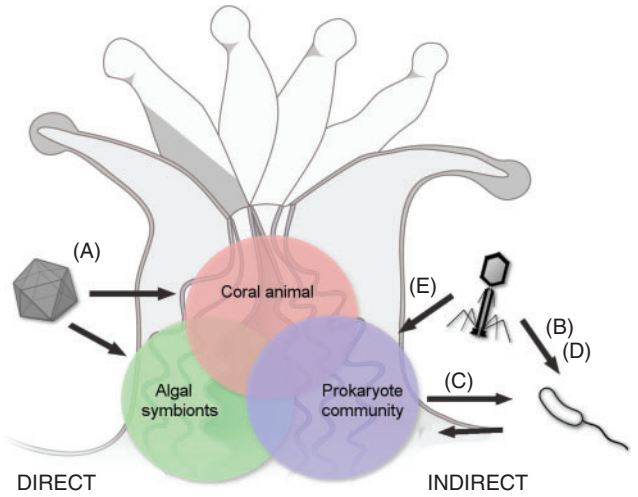

Figure 1. Viruses in coral health and disease. Viruses could contribute to or interfere with disease pathogenesis, for example, through direct (labelled A) and indirect (labelled B–D) processes. (A) Direct processes include eukaryotic viruses that target either the coral animal or *Symbiodinium*, e.g. as suggested in the case of virus-induced coral bleaching and yellow blotch disease, the virus on its own would cause the disease, therefore a direct interaction. (B–D) Indirect processes include bacteriophages that interact with the prokaryote community, which then have a secondary influence on the coral animal or algal endosymbionts (*Symbiodinium* spp.). (B) A bacteriophage might increase the virulence of an infected bacterium though horizontal gene transfer of virulence genes, which then causes a disease in the coral. In addition, bacteriophages may infect and lyse pathogenic bacteria, reducing the impact of a disease (C) as part of the coral microbiome, or (D) external from the coral holobiont, e.g. applied manually in phage therapy. (E) A bacteriophage may also interact with the coral prokaryote community and lyse a probiotic bacterium, which could open up a niche for a coral pathogen. Individual images publicly available for reuse with modification.

of *Symbiodinium* cells and has tentatively been linked to the abundance of virus-like particles (VLPs)[13]. Similarly, corals affected with white plague disease in the Caribbean have shown increased numbers of single-strand DNA viruses[14]. For both diseases, associated viruses still need to be isolated to investigate their causality using methods such as Koch's postulates.

Viral lysis (disintegration of infected cells) of *Symbiodinium* may be responsible for some instances of coral bleaching (Figure 2). A distant cousin of the dinoflagellate *Heterocapsa circularisquama* RNA virus, first detected with metatranscriptomics[16], has recently gained attention for its potential role in coral bleaching (reviewed in Thurber *et al.*[17] and Sweet and Bythell[18]). Transcripts of the ssRNA virus were shown to be present at high abundance in a heat-sensitive *Symbiodinium* culture, while they were barely detectable in a conspecific heat-tolerant culture, suggesting *Symbiodinium* and perhaps coral thermal tolerance is linked to the presence of this virus[19]. In order to progress the research in the field, PCR primers have been designed to assess presence and diversity of the ssRNA virus; these primers can potentially be modified for virus quantification during *in situ* coral bleaching events[20].

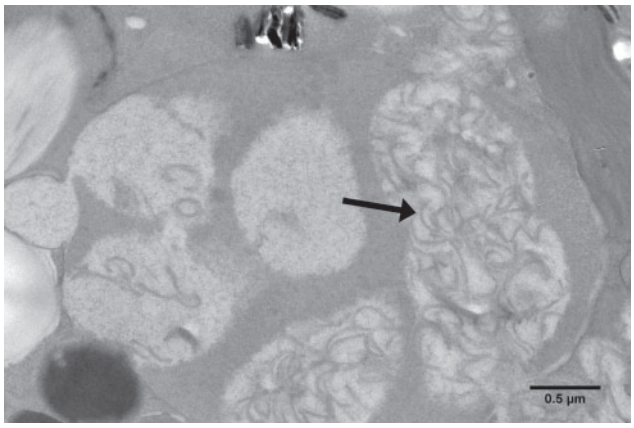

Figure 2. Transmission electron microscopy image of a cultured *Symbiodinium* cell, thin sectioned. The chromosomes of an untreated control strain of *Symbiodinium* C1 were degrading while showing the presence of unknown filamentous virus-like particles. Chromosomes in the image are light grey circular shaped. Filamentous virus-like particles are indicated by the arrow. *Symbiodinium* strain was cultured at the Australian Institute of Marine Science. Image courtesy: Karen Weynberg[15].

The potential roles of bacteriophages in coral disease

The mechanisms by which lysogenic and lytic bacteriophages interfere with or contribute to coral disease pathogenesis are primarily indirect, i.e. bacteriophages on their own do not influence the coral animal or *Symbiodinium*, but infect bacteria, which then secondarily influence coral health.

After infection of a target bacterium, the lysogenic stage refers to the integration of the bacteriophage genome into the bacterial host genome as a prophage. Bacteriophages may increase the virulence of a bacterial pathogen after establishing lysogeny and transferring new genetic material into the host bacterium. For example, the pathogenicity of the bacterium *Vibrio cholerae* primarily depends on infection by a lysogenic bacteriophage (CTXphi). The bacteriophage transfers genes that encode for one of the primary virulence factors, in this case the cholera toxin (CT), and converts *V. cholerae* from a non-pathogenic to a pathogenic strain[21]. Lysogenic conversion has been suggested to also increase the virulence of *Vibrio coralliilyticus* (Figure 1*B*), because parts of the bacterium's virulence factors that are linked to the coral disease white syndrome and coral bleaching are arranged similarly to the pathogenicity islands of the *V. cholerae* prophage[22].

Other lysogenic bacteriophages persist over extended periods of time until a trigger induces a lytic cycle, e.g., an increase in temperature or UV radiation. The lytic stage is characterised by the replication of bacteriophages within the bacterial host, which results in lysis of the host cell and release of newly produced bacteriophages[23]. For instance, traces of bacteriophages were detected in the CRISPR arrays within the genomes of cyanobacteria,

Roseofilum reptotaenium and *Geitlerinema* sp., two species dominating the black band disease mat in terms of biomass[24]. These findings suggest the cyanobacteria are regularly infected by bacteriophages and that phages may play a role in the disease development[24].

Viruses may also have positive effects on coral health, such as purely lytic bacteriophages[25]. Specific bacteriophages that target pathogenic bacteria may form part of the natural coral microbiome and confer some disease resistance by preventing bacteria from excessive proliferation (Figure 1*C*)[26]. Lytic bacteriophages have been applied successfully in lab-based phage therapies for the treatment of several bacterial coral diseases, e.g., white syndrome caused by *Vibrio coralliilyticus* strains[27,28]. The promising potential of phage therapy to treat a coral disease has been showcased, for instance, through the effective mitigation of white plague-like progression and transmission to other corals, during both a seven-week field experiment[29] and a 21-day laboratory experiment[27].

Conclusion and progress

Although viruses might contribute key aspects to coral bleaching and diseases, our understanding of this field of research is still scant. Even less is known about the functional contribution of viruses to coral health[18]. The current scarcity of coral virus-related studies can be linked to scientific challenges associated with environmental virus research and the difficulty to distinguish between causality and correlation of viruses with a coral disease. In order to overcome these issues, future research should establish coral host-virus model systems and consider versatile research approaches. For instance, relevant hosts for establishing virus cultures are *Symbiodinium* to investigate coral bleaching, *R. reptotaenium* for black band disease virulence models, and *V. coralliilyticus* for white syndrome virulence models. Multifaceted research approaches should include (1) viral metagenomics to characterise and describe virus communities in field-collected corals[30], (2) flow cytometry for virus enumeration[31], (3) liquid and plaque assays to isolate bacteriophages[32], and (4) bioinformatic pipelines that are designed for virus sequence data[33]. Research over the next decade will likely solve some of these issues and shed more light on the ecological importance of viruses in coral holobiont functioning. This will hopefully provide new ways to manage coral diseases on the reef.

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Swimming in the sea: chemotaxis by marine bacteria

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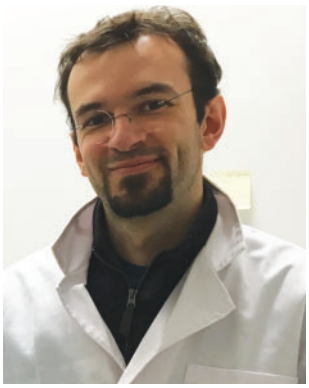

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Like many organisms, bacteria regularly inhabit environments characterised by spatiotemporal heterogeneity in the availability of resources required for growth and energy generation, meaning they must either tune their metabolism to prevailing conditions or have the capacity to migrate to favourable microenvironments[1]. To achieve the latter, bacteria measure their resource landscape and suitably direct their locomotion using a behaviour called *chemotaxis*, which is the ability to guide movement up or down chemical gradients. The capacity to perform chemotaxis is widespread across the bacterial domain, although most of our understanding of this phenotype is derived from enteric bacteria[2,3]. In the ocean, marine bacteria are often motile[4], and in fact capable of much higher swimming speeds[5] and chemotactic precision[6] than these enteric models for chemotaxis[2]. Here we discuss the underlying motives and purposes for bacterial chemotaxis in the ocean, by noting that marine bacteria experience a surprisingly heterogeneous chemical seascape[7,8], whereby chemotaxis can provide substantial fitness advantages and even influence large-scale processes including marine ecosystem productivity, biogeochemical cycling and disease.

Chemotaxis

Motile bacteria propel themselves by rotating helical flagella driven by molecular motors[2,3]. Chemotaxis by these motile cells is achieved through the constant measurement of local chemical concentrations using trans-membrane chemoreceptors, while a complex signal transduction network interprets this information, allowing cells to detect chemical gradients and regulate motility accordingly[1–3]. This chemotactic behaviour ultimately allows bacteria to swim toward favourable chemicals and away from noxious substances (Figure 1). Chemotaxis is one of the best-described sensory systems in biology, with a highly developed understanding of this behaviour acquired from well-defined model organisms, such as *Escherichia coli*[2]. The importance of chemotaxis is typically