



ORIGINAL RESEARCH

First Insights into the Viral Communities of the Deep-sea Anoxic Brines of the Red Sea



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Received 29 March 2015; revised 9 June 2015; accepted 29 June 2015
Available online 31 October 2015

Handled by Fangqing Zhao

KEYWORDS

Viral diversity;
Metagenomics;
Brine–seawater interface;
Caudovirales;
Red Sea

Abstract The deep-sea brines of the **Red Sea** include some of the most extreme and unique environments on Earth. They combine high salinities with increases in temperature, heavy metals, hydrostatic pressure, and anoxic conditions, creating unique settings for thriving populations of novel extremophiles. Despite a recent increase of studies focusing on these unusual biotopes, their viral communities remain unexplored. The current survey explores four metagenomic datasets obtained from different **brine–seawater interface** samples, focusing specifically on the diversity of their viral communities. Data analysis confirmed that the particle-attached viral communities present in the **brine–seawater interfaces** were diverse and generally dominated by **Caudovirales**, yet appearing distinct from sample to sample. With a level of caution, we report the unexpected finding of Phycodnaviridae, which infects algae and plants, and trace amounts of insect-infecting Iridoviridae. Results from Kebrit Deep revealed stratification in the viral communities present in

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Peer review under responsibility of Beijing Institute of Genomics, Chinese Academy of Sciences and Genetics Society of China.

<http://dx.doi.org/10.1016/j.gpb.2015.06.004>

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the interface: the upper-interface was enriched with viruses associated with typical marine bacteria, while the lower-interface was enriched with haloviruses and halophages. These results provide first insights into the unexplored viral communities present in deep-sea brines of the **Red Sea**, representing one of the first steps for ongoing and future sampling efforts and studies.

Introduction

The development and widespread use of molecular-based methods in environmental microbiology revealed that microbes dominate our planet. Ocean-dwelling bacteria are estimated to outnumber stars in the universe by several orders of magnitude (total numbers are 10^{29} and 10^{21} , respectively) [1], with even higher values for viruses. Viruses are the most abundant biological entities on Earth (10^{30} for total number of prokaryotic viruses or phages) [2], and harbor the second greatest biomass, after prokaryotes [3,4]. Furthermore, they are crucial ecological factors, which affect microbial diversity, population dynamics, and the genomes of their hosts [5]. Their impact extends from influencing microbial evolution, to playing an indirect but significant role in the Earth's biogeochemical cycles [5–7].

Despite a historically stronger focus on medically-relevant viruses, recent years novel technologies brought forth increasing activities in the field of environmental virology, with multiple studies centered in marine and aquatic environments, as well as several extreme environments. Such ongoing efforts have led to the discovery and description of multiple new viruses and increased our understanding of viral communities (*e.g.*, [2,5,8–10]). Since viruses lack a shared universal phylogenetic marker such as universal ribosomal DNA (rDNA), genetic diversity of environmental viral communities is increasingly assessed through metagenomic sequencing, which provides more and more information about viral diversity and evolution [11,12]. Nonetheless, metagenomic data have shown that we have yet to discover the majority of viruses present in the environment: over 70% of the genes in the oceanic viral fraction cannot be associated with known viruses [7]. Furthermore, studies on extreme environments, which include a few metagenomic-based surveys (*e.g.*, [13,14]), have uncovered that hypersaline environments host the highest viral densities reported for aquatic systems [15], yet still very little is known about them.

The deep-sea brines of the Red Sea include some of the most extreme and inaccessible environments on Earth, combining high salinities with increase in temperature, heavy metals, hydrostatic pressure, and anoxic conditions [16]. The microbiology of these brines received considerable attention in the last few years, with studies using an array of culture-dependent [17–21] and molecular-based approaches, including metagenomic studies [22–33]. Nonetheless, the viral communities of these extreme biotopes remain unexplored.

This study makes use of four metagenomic samples, obtained from different brine–seawater interfaces from the Red Sea, providing the first, though partial, insights into the viral diversity and community structure present in these environments.

Results and discussion

In this study, we used the DMAP's comparison module, associated taxonomic browsing, and filtering capabilities to explore the viral subset of annotations of four metagenomic samples (AT, DD, KU, and KL) obtained from the brine–seawater interfaces of different depths in the Red Sea. The resulting taxonomic comparison of these samples showed the relative proportions of bacteria, archaea, and viruses (**Table 1**). While the number of genes associated with viruses might seem relatively low, it should be noted that our source data refers only to reads recovered from the 0.1- μm fraction (*i.e.*, particle-attached or from infected cells). The ensuing analysis is therefore restricted to only part of the total viral diversity present in these environments.

General viral diversity

Analysis of the metagenomic datasets confirmed that the particle-attached viral communities present in the brine–seawater interfaces were diverse and, despite some similarities, distinct from sample to sample (**Figure 1**). This is likely a reflection of differences in microbial community profiles, which are specific to each location, and imparted from changes in physicochemical conditions [16,26,30–33].

As a general trend, we observed a clear dominance of dsDNA viruses, which accounted for 45%–85% of the viruses detected (**Figure 1**). Further scrutinization indicated that dsDNA viruses are mostly Caudovirales (**Figure 2**). Caudovirales can be further classified as Syphoviridae, Myoviridae, and Podoviridae, while various proportions of them remained unclassified (**Figure S1**). Caudovirales are tailed bacteriophages, which are known to dominate in marine and other aquatic environments [9,34,35].

Other viral families were detected at much lower abundances, including the unexpected Phycodnaviridae (4%–6%), which infect algae and plants, and trace amounts of insect-infecting Iridoviridae (<1%). These viral taxa are likely derived from the particles originated away from the brines, including host lysis products that act as virus scavengers [5]. We hypothesize that these particles sink through the water column, and are eventually trapped and accumulated in the density gradient of the brine–seawater interface. Viruses (and DNA, in general) have been previously reported as having longer-term stability, and can be preserved in such deep-sea brines [36–38]. Similar observations have been reported for other marine locations, particularly when transitioning from oxic to anoxic conditions [9]. Detection of these unexpected viral taxa would thus be the result of a “pickling” effect, rather than reflecting the presence of specific hosts in close proximity to the brines.

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