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How viruses control microbial ecosystems

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Viruses are the most abundant nucleic acid containing biological parcel on earth, being ten times more than bacteria and archaea [1]. Most are phage (infect bacteria), have a genome of DNA or RNA, and are encapsulated in a protein coat at concentrations of 10^{10}/L in aquatic environments. The planet holds 10^{31} viruses in the oceans alone [2] and their biomass equals 200 Mt of carbon or 75 million blue whales [1]. Stretched end-to-end they would span 10 million light years [1]. They are obligate parasites and without doubt a pervasive influence on microbial ecosystems.

Host-viral isolates from the environment

Have you ever wondered why some prokaryotes are hard to isolate from the environment? One reason cultures may sometimes appear fastidious is that you are dealing with a complex set of viral-host interactions. As cited in the review of Weinbauer [3], at least half of all the bacteria isolated by Ackermann and DuBow [4] were lysogenic (phage genome within host). Similar percentages (40% to 52%) have been reported for bacterial isolates from marine and estuarine environments [5,6] and most recently 30% to 44% of the bacteria isolated from soils [7]. Hence, lysogeny in bacterial hosts is prevalent, at least for isolates. Lysogeny can confer an environmental robustness on the host over its uninfected counterpart in a mutually beneficial relationship [3]. However, the resident bacteriophage can move to a lytic (phage multiples and causes lysis of host) life cycle if the host becomes stressed, for example during increased temperatures that cause physiological stress on the host [3]. In my experience, sampling bacteria from a natural environment into a sterile medium can be enough to metabolically stress the host and cause viral lysis (prophage induction) of the host. These populations do not end up in the culture collection. By default, culturing methods select against lysogenic bacterial populations. Changes to the microenvironment that metabolically stress the host can cause the viruses to move from a lysogenic to a lytic life cycle where the virus sacrifices its host to ensure viral survival – the 'SOS' response.

Viral influence on biogeochemical cycles

Today, research in viral ecology [8] is where we were in the 1980s with bacteria when Azam *et al* [9] first described the microbial loop; the beginning of our acceptance of the critical role of bacteria and archaea in biogeochemical cycles [10-12]. With developments in the direct measurement of viral dynamics in ecological processes [13] we see viruses altering prokaryotic diversity, community function, structure, mass, and energy transfer between trophic groups.

Via the uptake of dissolved organic carbon (DOC), heterotrophic bacteria mediate total carbon and nutrient flux of freshwater and marine ecosystems [14,15]. In the oceans, bacterioplankton production is supported by the flow of organic carbon from predominantly phytoplankton, while terrestrial organic carbon inputs underpin freshwater ecosystems [11,16].

My study of a freshwater/estuary ecosystem (Bremer River, Ipswich, Queensland) suggested that bacterial production is converted into DOC through viral lysis. Heterotrophic bacterial production, bacterial and viral abundance and primary production was measured. This microbial ecosystem was 'heterotrophic' i.e. dominated by heterotrophic production [17] and there were ten times more viruses than bacteria. The viral (virus-like particles; VLP) to bacterial ratio was high (Figure 1) compared to marine oligotrophic environments, suggesting high infection and lysis rates. High numbers of VLP followed bacterial abundance and growth rate, suggesting that there was a bacterial-viral loop inside the microbial loop described by Azam *et al* [9].

Viral lysis products (e.g. DOC) are available for bacterial growth [18]. DOC has been shown to contain remains of bacteria [19]. In my study [17] viruses lysed the bacteria and the resulting DOC was then used by other bacteria that viruses further lysed generating more DOC and the cycle continued in an inner bacterial-viral loop (Figure 2). Ultimately in each pass of the loop DOC was respired to the atmosphere as CO_2. This bacterial production was not passed up the food chain to higher trophic groups. Viral infection and the lysis of bacteria released DOC that other bacteria subsequently used. This process was possibly responsible for removing much of the dissolved organic matter from that microbial ecosystem. This was also consistent with the observed high bacterial growth rates and constant bacterial biomass. A similar conclusion has been reached for marine biogeochemical cycles; viral infection and lysis limited the microbial production passing up the food chain [13,20].

Overall viruses tend to increase heterotrophic bacterial production and respiration (by 33%) while reducing production in higher trophic levels [13]. This is further evidence of viruses controlling organic carbon flow in subtropical environments as they again divert dissolved organic carbon from higher trophic groups back into the atmosphere as CO_2 by increasing heterotrophic bacterial respiration. In subtropical/tropical freshwater ecosystems, cyanophage can also control cyanobacterial production [21] with between 13 – 46% of bacteria mortality being viral [22].

The viral influence on microbial ecosystems of the oceans extends to the atmospheric carbon budget and may profoundly influence global warming. Phytoplankton take up nutrients and CO_2 through photosynthesis. Some of the organic matter created is cycled through the food web in the upper ocean and some sinks. Some of this carbon is re-mineralised back to CO_2 while a fraction sinks as particulate material to the deep interior of the oceans and the sea floor; this is the 'biological pump'. Without this biological pump locking up carbon in the deep interior of the oceans (albeit small), the concentrations of atmospheric CO_2 would be three times higher [23]. The rate of viral lysis of phytoplankton communities influences the global carbon budget by making dissolved organic carbon (DOC) (primary production) available for bacterial respiration, ultimately returning CO_2 to the atmosphere rather than sinking to the deep ocean interior [1]. Viral lyses converts as much as 26% of the primary production to DOC [24].

With Jeff Argo, a PhD student under my supervison, I have observed temperature increases can cause viruses to change from lysogenic to lytic viral life cycles. We have been growing two cyanobacteria species *(Microcystis aeruginosa* and *Cylindrospermopsis raciborskii)* in culture for two years. After a 3°C temperature rise in the 'constant' temperature room, both species died. The media filled with cell debris and viruses as the viral-host relationship changed; the viruses moved from a lysogenic to a lytic life cycle as their hosts became metabolically stressed with the increased room temperature. As the oceans become warmer, will we see increased viral lysis of phytoplankton and less CO_2 returned to the deep ocean, further compounding the global warming affect? We already see prophage induction in the warmer oceans of the world [25].

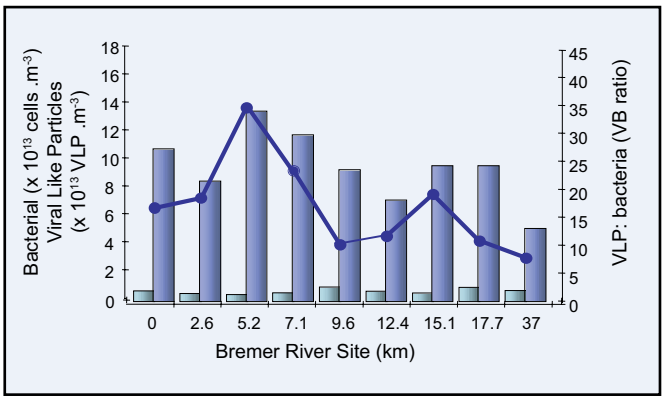

Figure 1. Bacterial (grey) and viral (blue) (virus-like particles, VLP) abundance and the ratio between the two (line) along the Bremer River, Ipswich Queensland. Distances are from the junction with the Brisbane River.

Prokaryote diversity, function and community structure – preventing the hostile takeover

Phage can affect the diversity of bacterial and algal communities by 'killing the winner', to control competitive dominance by preventing the most prolific populations taking over a

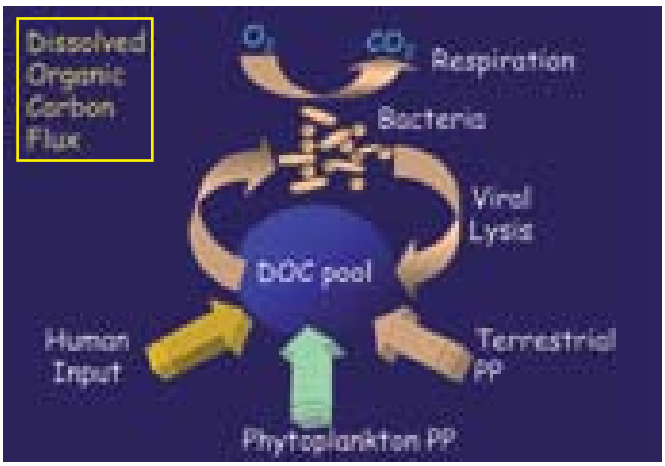

Figure 2. Viruses lyse bacteria; other bacteria then use the resulting DOC these bacteria are further lysed generating more DOC as part of an inner bacterial-viral loop. In each pass of the loop DOC is respired to the atmosphere as CO_2. This bacterial production is not passed up the food chain to higher trophic level. PP = Primary Productivity.

community [3]. As a population increases in number it is more likely to encounter an infective virus, become infected and lyse [26]. Thus, viruses are controlling species diversity in microbial ecosystems. Hennes *et al* [27] show that even low abundances of viruses in natural aquatic viral communities can control microbial community structure and diversity. Indirectly viruses may also promote bloom occurrence by regenerating DOC or shifting the composition of microbial communities [28].

Viruses control the function of prokaryotic populations. They have a deterministic role in the host genetic make-up and alter the host's genetic information while co-evolving with their host bacterium. Most bacterial pathogens contain prophage DNA integrated into the bacterial DNA that codes for virulence factors [29,30]. *Vibrio cholerae* is a good example of a host relying on the infection of a virus to carry its toxin gene [29]. The phage movement of functional bacterial genes in the environment is just beginning to be explored. Viruses mediate gene transfer and expression in environmental bacterial populations, they use the host to assist diversification of viral community but lack conserved genetic sequences [31].

Conclusion

Most research into environmental viral ecology has been in the marine environment over the last fifteen years and more recently in freshwater with little in soil. Despite this inequality, strong evidence exists to say that viruses can control microbial ecosystems in aquatic environments and this is likely true for soil, through some key processes.

- Natural viral populations are likely major controllers of microbial diversity and play a role in preventing any single populations dominating a community.
- Every second bacterium is infected and lives in a lysogenic life cycle that makes the host bacterium more robust, in a mutually beneficial relationship, until the host is metabolically stressed.
- Viruses influence biogeochemical cycles on micro and global scales by preventing primary and secondary bacterial production passing to higher trophic levels; cell production is respired and returned to the atmosphere as CO_2 recycling inorganic nutrients (e.g. nitrogen and phosphorus) at the same time.
- Viruses alter diversity and function of microbial communities by exchanging functional genes within and between populations, bacteria and viruses are coevolving
- Viral diversity evolves with its host as the viral-host interaction is continuously selecting the best infection-defence strategy.

Acknowledgement

I am most grateful to Jeff Argo (Griffith University) and two anonymous referees for their valuable contributions.

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