Diversity and activity in marine prokaryotes

Arrieta López de Uralde, Jesús Maria

Summary

Life on Earth depends on the endless recycling of elements as matter and energy are required to sustain life. The prokaryotes (Bacteria and Archaea) are the masters of the trade of life. After all, they were already responsible for the major biogeochemical cycles 3,000 million years ago, long before other groups of organisms emerged on this planet. Thus, it is not surprising that the phylogenetic and metabolic diversity of prokaryotes is so enormous compared to that of eukaryotes. This metabolic diversity allows prokaryotes to carry out amazing tasks. They are the basis of the food chain around hydrothermal vents on the sea floor where the only energy sources are "noxious" chemicals. Prokaryotes are also the little Cinderellas that clean the mess left behind by the rest of the living organisms bringing back into the biogeochemical cycles the stuff that nobody else can use. Like Cinderella, prokaryotes are often despised. A lot has been written in the last years about the importance of preserving the biodiversity of large, visible organisms. However, when it comes to prokaryotes the public awareness shifts towards disease, food spoilage, and unpleasant smells. Yet, the survival of all the large organisms, including us, depends on the metabolic abilities of prokaryotes. Thus, understanding the mechanisms driving prokaryotic diversity and activity is crucial to comprehend the functioning of ecosystems and ultimately the factors controlling our own survival.

Increased levels of harmful ultraviolet (UV) radiation reaching the Earth's surface have been reported over the last decades. Prokaryotes are especially sensitive to UV radiation. Physical considerations suggest that due to the small size, they cannot produce "sun-blocking" substances to prevent DNA and protein damage. However, prokaryotes are very good at recovering from UV-stress. If their DNA is damaged, they can replace a damaged DNA strand with a new copy based on the complementary strand (dark repair). Some prokaryotes can even reverse the chemical structure of damaged nucleotides using special enzymes and solar energy (photoenzymatic repair). Although physical considerations suggest that prokaryotes cannot prevent damage by UV radiation, our results show large differences in sensitivity to UV radiation among different bacterial species. Huge differences were observed in the ability to recover from UV-stress using either dark repair or photoenzymatic repair. These data suggest that exposure to UV radiation could easily produce shifts in the species composition of marine prokaryotic communities.

The oceans cover approximately 70% of the Earth's surface and harbor large numbers of very busy prokaryotes. For example, one milliliter of North Sea water contains from several hundred thousands to millions of prokaryotes, which can occasionally grow as fast as to double their numbers within a few days. Phytoplankton are photosynthetic organisms living in the surface of the ocean. In the North Sea, phytoplankton blooms (massive growth of tiny algae) are often so huge that the human eye can detect a change in the color of the sea. Sometimes these blooms produce so much organic material that impressive piles of organic-rich foam can be spotted on the beaches. A major fraction of these materials consists of long polymeric

molecules like proteins and carbohydrates. Prokaryotes use enzymes to hydrolyze (break down) these long molecules outside of the cell before they can take them up in the same fashion as we use enzymes to digest our food in the stomach before we can absorb it in the intestine. One of these hydrolytic enzymes, β-glucosidase is responsible for splitting sugar chains similar to those found in cellulose into glucose molecules that can be easily utilized by prokaryotes. We detected high levels of prokaryotic β-glucosidase activity after the collapse of a phytoplankton bloom during the spring of 1999, suggesting the release of large amounts of carbohydrates. While this is the normal response of the prokaryotic community, it was unclear whether this was a general response or whether some specific members of the prokaryotic community were responsible for the observed increase in β-glucosidase activity. We developed a highly sensitive technique (capillary electrophoresis zymography) that allowed us to produce a "fingerprint" of the β-glucosidases present in a seawater sample. With this technique, we could detect how many different β-glucosidases (probably produced by different species or at least by different genes) were present in the sample, as well as the relative amount of each of them. Previous findings suggested that the diversity of β-glucosidases in marine systems was very low, probably only 1 or 2 different types of β-glucosidase. Surprisingly, our results showed that 11 different β-glucosidases were expressed over the two months study and up to 8 could be found concurrently in the same sample. Moreover, the changes in the β-glucosidase "fingerprint" could be related to changes in the species composition of the bacterial community. The collapse of the phytoplankton bloom induced fast growth of a few bacterial species with high β-glucosidase activity resulting in a decrease in bacterial diversity.

Phytoplankton take up CO_2 from the water column and use light to convert it into organic compounds in the same way as terrestrial plants. There is constant exchange of CO_2 between the surface layers of the ocean and the atmosphere. If CO_2 is removed from surface waters by phytoplankton, more CO_2 enters from the atmosphere. If phytoplankton dies in the surface ocean, herbivores and bacteria will respire the major fraction of this organic carbon, producing CO_2 which can go back again to the atmosphere. However, some of this carbon will eventually escape the food chain sinking into deep layers of the ocean or to the sediment where it can be preserved over geological time scales. The net effect is that this carbon will be removed from the atmosphere for a very long time. This is a natural process known as the "biological pump".

Iron limitation is the major factor preventing phytoplankton blooms in certain areas of the ocean like the North and Equatorial Pacific and the Southern Ocean. It has been shown that iron fertilization promotes the growth of large, fast-sinking algae in these regions. Therefore, it has been hypothesized that iron fertilization could be used to enhance the "biological pump" leading to the removal of vast amounts of CO_2 from the atmosphere, which in turn, would help reduce global warming. However, several scientific issues need to be resolved before this idea can be taken seriously and some of them concern prokaryotes. It is assumed that after iron fertilization, only the number of large algae will increase significantly, while the abundance of prokaryotes and small algae will be kept low by microzooplankton (single-celled animals able to eat prokaryotes). It is also assumed, although there are contradicting reports on this, that prokaryotic activity is not directly limited by iron in these waters. Therefore, the impact of prokaryotic production and respiration on organic carbon would not increase a lot after iron fertilization. However, if prokaryotic respiration would increase upon iron fertilization, either by an increase in abundance or activity or both, the estimated enhancement of the "biological pump" would be reduced and so would be the usefulness of iron fertilization. Additionally,

to unpredictable consequences. We joined an iron enrichment experiment in the Southern Ocean during the austral spring of 2000 in order to check the validity of these assumptions. An experienced group of "ocean gardeners" found a suitable spot, an eddy of approximately 150 km diameter, containing high concentrations of all the major nutrients except iron. An area of about 50 km^2 around the center of the eddy was fertilized with iron sulfate, and re-fertilized every 8 days to keep the concentrations of dissolved iron high. Samples were taken both inside and outside of the fertilized patch for three weeks. As expected, iron fertilization produced a phytoplankton bloom and a shift towards larger phytoplankton species. Our data on prokaryotes do not support the previously mentioned assumptions, prokaryotic abundance almost doubled inside the fertilized patch as compared to the surrounding waters. Prokaryotic activity greatly increased in iron fertilized waters, either by the direct effect of iron fertilization or indirectly by the increased carbon and energy supply associated with the growth of phytoplankton. Our capillary electrophoresis assay showed both, an increase in the activity of those β-glucosidases already present prior to the fertilization and the production of new β-glucosidases that were barely detectable outside the fertilized patch. No major changes were observable in the species composition of the prokaryotic community. Our data suggest that prokaryotic communities in the Southern Ocean are well adapted to episodic iron input. According to these results and other work in progress, the hypothesized magnitude of carbon sequestration processes due to iron fertilization may be much smaller than expected.

It is important to realize that these results cover only the initial response of prokaryotic communities. The effects of long-term iron fertilization and the subsequent increases in productivity may have a larger impact on the bacterial community structure. Many studies show that productivity is one of the major factors controlling plant and animal diversity in natural communities. Different patterns have been observed, sometimes diversity increases or decreases with increasing productivity and frequently maximum diversity is observed at intermediate levels of productivity. If such a relationship could be found for prokaryotes, it could help predicting the effects of changing productivity. We collected samples across the Atlantic Ocean covering a wide range of latitudes (>9,000 km from the northernmost to the southernmost location) and depths (from surface to ~3,880 m) in order to determine the possible relationship between prokaryotic productivity and diversity. These data indicate that total prokaryotic diversity is highest at intermediate productivity values. Such a "hump-shaped" relationship between productivity and diversity has been often observed for larger organisms. Different trends were detected in different habitats. Diversity increased with increasing productivity in deep waters but the opposite was observed in surface waters. Moreover, the two major groups of prokaryotes (Bacteria and Archaea) showed different trends. While bacteria were responsible for the overall "hump-shaped" pattern, a continuous increase in diversity was observed for Archaea with increasing productivity.

I hope that this bunch of disparate studies will prove my point. Greater insight into the dynamics of prokaryotic communities can be gained by combining metabolic activity measurements with diversity assessments. This might seem obvious to many, but it is seldom done in microbial ecology.