

The Sea as a Gene Pool

The oceans are full of bacteria. Outwardly, they all look much the same, but there are many different species living a variety of ways of life. This has led **Hanno Teeling**, **Bernhard Fuchs** and **Frank Oliver Glöckner** from the **Max Planck Institute for Marine Microbiology** in Bremen to analyze bacterial diversity in the oceans with the aid of metagenomics. To do this, they first throw the whole bacterial genome into one pot, then decode the DNA molecules and sort the genetic mix back into individual bacterial groups.

Millions of bacteria live in a single liter of seawater off of nutrient-rich coasts, such as the popular beaches of the North and Baltic seas. While hardly any differences can be seen under the microscope, the DNA rings of their genomes reveal their various ways of life.

TEXT **NILS EHRENBERG**

A beaker of seawater looks pretty dull at first glance. It is only under a microscope that a distinct universe of shapes and colors is revealed – the world of the tiniest micro-, nano- and picoplankton. Diatoms and radiolarians, in particular, really stand out under the microscope, with an endless variety of geometric shapes: from circles, ovals, triangles and squares to intricate filigree stars.

“Although the larger single-celled organisms are often spectacular, the vast majority of plankton consist of smaller bacteria,” says Frank Oliver Glöckner, head of the Microbial Genomics and Bioinformatics Research Group at the Max Planck Institute for Marine Microbiology in Bremen. “While these bacteria are virtually indistinguishable under the microscope, they actually show even greater differences in their ways of life.”

The species diversity of plankton has long been a puzzle to scientists, standing as it did in direct contradiction to the accepted ecological theories. In contrast to tropical rain forests with their many ecological niches, each offering different conditions for life, the oceans offer only huge, seemingly unvarying bodies of water with no clear

»» The genome is the only reliable distinguishing feature between bacterial species.

spatial structure. An ecological niche is defined as all the factors in the living and inanimate environment that an organism needs to survive.

According to the competitive exclusion principle, species that compete for the same ecological niche can't co-

exist permanently: sooner or later, the stronger species will win out and displace all of its weaker competitors. So how can a uniform habitat like the ocean harbor such great species diversity? Why isn't the plankton dominated by a few species?

"We want to resolve this plankton paradox through our research," says Frank Oliver Glöckner. "But before doing this, we need to get a proper idea of the full biodiversity of bacterial plankton. We want to know what species there are, how common they are and what function they have in the ecosystem."

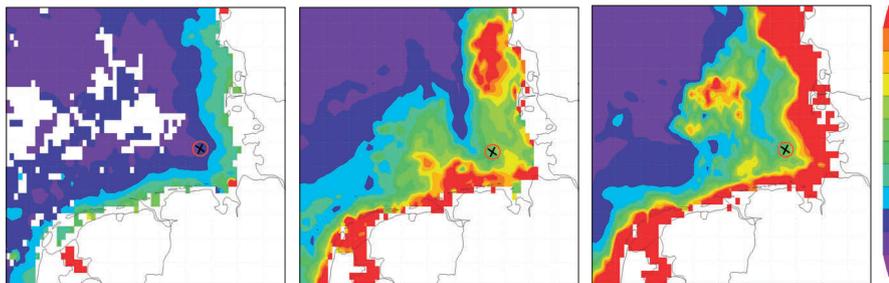
To do this, the scientist at the Bremen-based Max Planck institute is relying on the newest genomic and computer-based techniques. As the bacteria look so similar under the microscope, differences in their genomes are the only reliable identification markers for individual species.

EXPERIMENTS, MODELS, GENETIC ANALYSES

At the institute in Bremen, Glöckner is working with researchers from various disciplines, including bioinformaticians, geneticists, microbiologists and ecologists. Ecologist Bernhard Fuchs is conducting experiments to investigate what the bacteria eat, as well as how they stick onto each other or to algae. His colleague Hanno Teeling has a very different job: "Computer analysis of a bacterial genome tells me what a cell might be capable of and what enzymatic tools it may be equipped with."

Precisely because of their contrasting viewpoints, the two scientists attach great importance to a mutual exchange of views. "I'm working on the computer with organisms I've never seen," explains Teeling, "and the single cell can become an abstract concept. Thanks to Bernhard Fuchs, I know whether the cells are really doing what I predicted with my data."

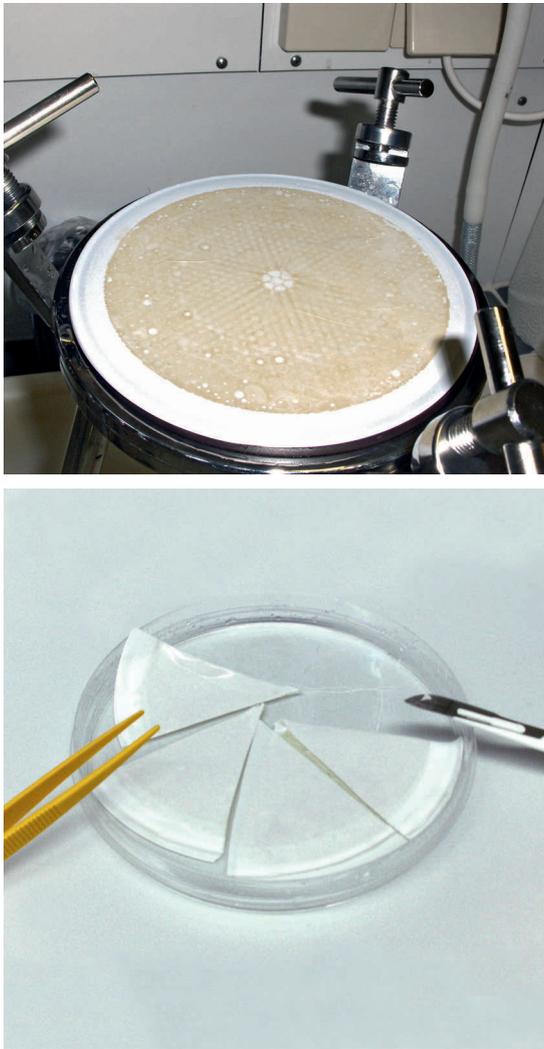
Working together with scientists at the Alfred Wegener Institute for Polar and Marine Research, Jacobs University Bremen and the University of Greifs-



above: Chlorophyll in the North Sea in February (left), March (center) and April 2009. The chlorophyll level is a measure of algal numbers and thus also of the productivity of a marine region. Blue areas indicate low algal concentrations, while red shows high ones. The cross marks the location of the island of Helgoland.

below: Helgoland's main island can be seen in the foreground, with the smaller island of Düne behind it. Situated in the channel between the two islands is the *Kabeltonne* marine station, where the researchers collected their samples.





Frank Oliver Glöckner filters the bacteria out of the seawater with the aid of a polycarbonate filter (top left). This filter is then cut into smaller pieces, from which the DNA is harvested for further analyses (bottom).

wald, Teeling, Fuchs and Glöckner studied an algal bloom in the North Sea and recently published their results in the journal *SCIENCE*. “As soon as the level of solar irradiation exceeds a certain level in the spring, single-celled algae such as diatoms can proliferate massively within a few days,” explains Frank Oliver Glöckner. This sort of algal bloom often ends as quickly as it began: the nutrients dissolved in the water are depleted, and growth is inhibited.

In the end, the algae are either eaten up by zooplankton – crustaceans about a millimeter in size – or are killed by viral infection. And this is the moment of glory for the bacteria, which fall upon this feast of algal remains.

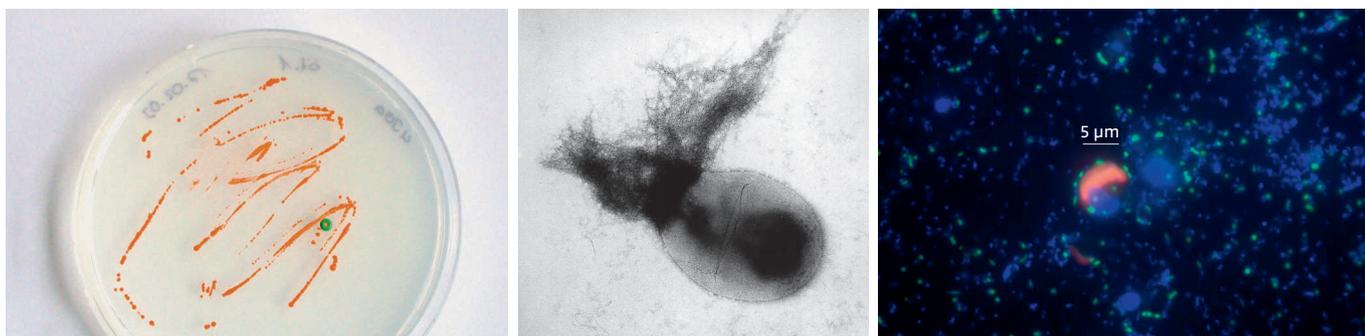
“The bacterioplankton changes dramatically after an algal bloom,” says Bernhard Fuchs. “We believe that this phenomenon is one aspect of the solution of the plankton paradox.”

WEEKLY MEEASUREMENTS IN THE NORTH SEA

The marine scientists collected the samples for their study at the permanent *Kabeltonne* research station run by the Biological Institute Helgoland, located in the narrow channel between the main island and the smaller Düne Island, a favorite of beachgoers. Since 1962, researchers have carried out weekly measurements of various environmental parameters off the island,

such as salt content and water temperature – data that, given its regularity, is unique in the world and is important not only to the Bremen team. In summer 2009, even Craig Venter, one of the pioneers of genome research, paid a brief visit to Germany’s only off-shore island to take samples for genome analyses, just like the Bremen team.

Throughout 2009, the scientists traveled to the research station twice a week, each time taking up to 500 liters of North Sea water from a depth of one meter. At the laboratories of the Biological Institute Helgoland, they then measured the salt content, the concentrations of such nutrients as phosphate, silicate and nitrate, as well as levels of the green plant pigment chlorophyll A,



left: Colonies of *Pirellula* bacteria from the Baltic Sea on a culture slide. The bacteria can utilize carbon and nitrogen compounds and break down organic material such as dead algae. They adhere to the algal remains with their thread-like fimbriae (center).
right: Flavobacteria (green) on algae of the species *Phaeocystis* (red).

a measure of the number of algae in the water. Finally, they separated the bacteria, algae and viruses.

Back at the Max Planck institute in Bremen, it was then up to Bernhard Fuchs to determine the exact composition of this bacterial population. Using fluorescence in situ hybridization (FISH), the ecologist sent DNA probes into the cells to bind to the bacterial ribosome and cause it to light up with the aid of a fluorescing pigment. Since every probe recognizes only the ribosome of a specific group of bacteria, Fuchs can make this group visible in the water sample and count the individual cells using an automatic microscope. A species-specific marker gene can also be used to identify individual species in the sample.

EXPLOSIVE PROLIFERATION

“By doing this, we were able to determine exactly how the composition of the bacterial population in the water changes during an algal bloom,” says the ecologist. According to their data, alphaproteobacteria dominate in the water during the time before the algal bloom. But as soon as the massive algal proliferation takes place over the course of a few days, there is a dramatic change in this community. Flavobacteria by the name of *Ulvibacter* begin to divide rapidly, quadrupling their cell count within a week.

Just a few days later – with the algae already decimated by hungry zooplankton and viral infection – the *Ulvibacter* population collapses suddenly, with *Formosa* flavobacteria taking their place. They, too, initially proliferate on a grand scale, but their numbers drop drastically just a week later.

“And so it goes, with one group proliferating explosively, perishing again after a few days, and then being replaced by another group,” explains Bernhard Fuchs. “It’s only a few weeks after the algal bloom that the situation settles down and returns to the alphaproteobacteria-dominated status.” During this phase, there is little or no detectable evidence of most of these short-term upstarts in the water.

How can this turmoil in the bacterial community be explained? “The bacteria’s genetic makeup determines how they react to environmental conditions,” says Hanno Teeling. “We therefore need to know which genes the various species possess and which of them are active in the various phases after an algal bloom.” For this reason, the scientists analyzed the full genetic material of the entire bacterial community in their water samples – that is, the metagenome. Using a computer program, Teeling first searches for genes in this mix, as not all DNA segments code for a protein.

To accomplish this, the analysis program has to solve a whole series of

problems. For one, the sequencing machines don’t deliver any complete genomes, but only DNA fragments. The computer thus has to account for the fact that only parts of genes are present. For another, the sequencing machines often make errors when decoding the DNA code.

DNA: LIKE GOES WITH LIKE

But the true artistry consists in assigning the individual DNA molecules from this varied and motley genomic mixture to the various bacterial species. “As you might expect, the DNA snippets don’t carry nameplates, so we don’t know which bacteria they belong to. Because of this, one of our programs carries out an extensive similarity analysis with the identified genes on the DNA molecules,” says Hanno Teeling.

For instance, the computer looks for the most similar known gene of a known bacterial species, or identifies in the DNA code sequence characteristic patterns that occur only in a certain group. Finally, it sorts the individual snippets together with the genes they contain into virtual piles. “In this way, we get a fairly accurate idea of which genes belong to a certain group of bacteria,” says Teeling.

The task of the scientists from Greifswald who were involved in the study was now to fish out the metaproteome

»» So far, climate change hasn't altered the community of bacterial species in the seas.

– in other words, all the proteins – from the samples and to identify them. Since proteins can be matched up with their genes using special algorithms, and the genes of the various bacterial groups had been identified thanks to the metagenome analysis in Bremen, the scientists were able to say exactly which protein belongs to which bacterial group.

The team headed by Teeling, Fuchs and Glöckner now had all the information it needed: the exact composi-

tion of the bacterial community in various phases before, during and after the algal bloom; the genetic make-up of the individual groups; the proteins and the temporal development of the environmental conditions. The scientists now collated all this data and established connections. "If, for example, a certain sugar is released from the algal remains after the bloom, and if a certain gene is simultaneously activated in a bacterial group, this is a strong indicator of the presence of a

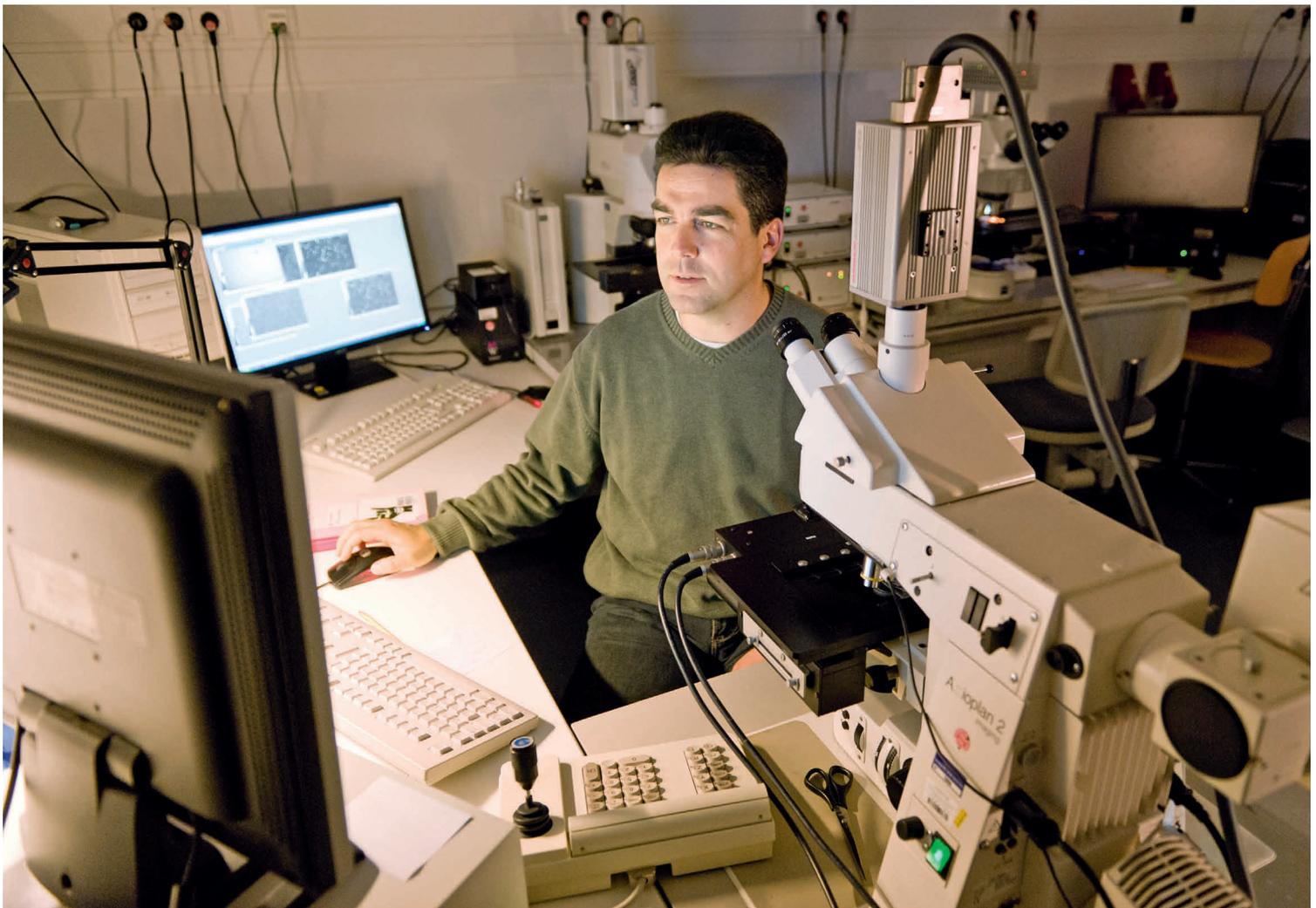
key protein that helps the group in question with the uptake of that sugar," says Hanno Teeling.

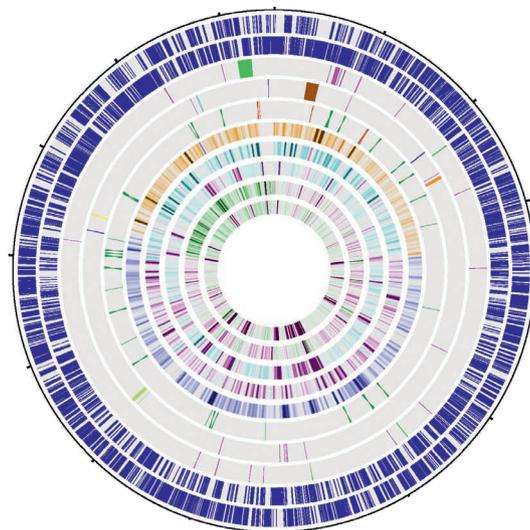
PROTEINS THAT BREAK DOWN SUGAR

The team from Bremen managed to identify an entire array of these key proteins, thus finally finding a solution to the puzzling dynamics of the bacterial population. The first big winner of the algal bloom, the flavobacterium

Photo: Bastian Ehl

Not an unusual workplace for an ecologist: Bernhard Fuchs studies bacteria with a fluorescence microscope.





Hanno Teeling reassembles the countless DNA snippets on his computer. In this illustration of the genome of *Congregibacter litoralis*, the two outer rings show potential genes, while the inner rings contain genes that code for various metabolic pathways.

Ulvibacter, produced large quantities of substances known as TonB-dependent transport proteins, which carry large molecules directly into the interior of the cell.

At the same time, the scientists found an increase in enzymes used to break down complex algal carbohydrates such as laminarin. In contrast, the latecomers among the winners show a predominance of transport proteins for short protein fragments, as well as transporters for the nutrient phosphate and for simple sugars.

NICHES SEPARATED BY TIME

“It seems that the bacteria adopt completely different feeding strategies,” explains Bernhard Fuchs. Some alpha-proteobacteria can use smaller nutrients very efficiently, as they specialize in low nutrient concentrations. For this reason, they predominate in the phases between algal blooms.

Ulvibacter, on the other hand, with the help of its enzymatic tool kit, can utilize the rich supply of algal remains directly after the bloom, and is the first to break down the long-chain carbohy-

drates. In contrast, the groups that follow concentrate on smaller and smaller molecules and utilize the leftovers. “It’s a bit like in the Serengeti – the lions come first, then the hyenas, the jackals, and finally, the vultures,” says Teeling.

Thus, the bacteria occupy ecological niches in the sea that are separated from one another, not by space, like in the rainforest, but by time. By specializing in various phases after the bloom, they avoid the competition. Suddenly, even the plankton paradox doesn’t seem quite so paradoxical, since the time separation maintains a high species diversity in the bacterioplankton.

“Our study is the first high time resolution analysis of a microbial community at the genus level,” says Frank Oliver Glöckner. “This was possible only through the extraordinary leap forward made by sequencing technology in recent years.” Indeed, today’s high-performance sequencers work in completely different spheres even than at the time of the Human Genome Project. “At that time, it took 10 years to sequence the entire human genome. Today, it could be done in 14 minutes,” says the biologist.

Glöckner is already working at full tilt to develop this technology further and to make his bioinformatics tools usable for other scientists as well. January 2012 saw the start of the international Micro B3 (Biodiversity, Bioinformatics, Biotechnology) project. Frank Oliver Glöckner is coordinating the consortium of 32 academic and industrial partners and has big plans. “In 2012, we want to organize an Ocean Sampling Day – on that day, water samples will be taken using standardized methods all around the world, and analyzed as they were in our own study. Our aim is to determine the size and the differences in bacterial biodiversity in the world’s oceans.”

Studies of this type could possibly explain the influence of climate change on marine bacterial communities. The consequences of the North Sea warming of 1.2 degrees Celsius since 1962 are already apparent on a large scale: cold-loving indigenous fish such as the cod are moving into the Norwegian Sea, whereas southern species such as striped red mullet and sardines appear to feel more comfortable in the German Bight.

However, the great migration among indigenous microbes appears not to have happened so far. "At the bacterial level, the warming has no effect at present; this species community is constant," says Bernhard Fuchs.

ENZYMES FOR INDUSTRY

However, the aim of the Bremen-based scientists isn't just to use metagenomics to study the composition of the ocean's species communities. The genes and proteins that they encountered during their analyses could also help in the development of new active substances or materials. In his research, Glöckner is cooperating closely with partners from the biotechnology sector: "Enzymes have many potential uses, because they speed up chemical reactions and lower energy consumption."

The Max Planck researchers are therefore working with various compa-

nies that are testing the new enzymes the researchers found in the bacterial genomes for possible use in the manufacture of drugs or detergents.

After all, many of the bacterial genomes, most of them consisting of 3,000 to 8,000 genes, have hardly been studied yet. For 30 to 40 percent of them, the experts know very accurately which proteins they code for and what exactly these proteins do. "These are primarily the enzymes in-

involved in basic metabolism, which every group of bacteria needs for survival. In another third of them, we have a rough idea of the group to which a gene belongs, for example whether it codes for a lipase that can break down fats," says Glöckner. But the last third are completely unknown and consist of genes whose existence is only suspected by the researchers. They are now seeking to unearth this genetic treasure. ◀

TO THE POINT

- The water of the oceans offers organisms few spatially separated habitats. Despite this, the seas are home to countless microorganisms (the plankton paradox).
- Marine bacteria adopt a variety of feeding strategies. They can occupy niches separated by time, thus avoiding competition with other species.
- After an algal bloom, the bacterial community is dominated by one species after another for short periods, each of them able to make optimum use of the available food supply.



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