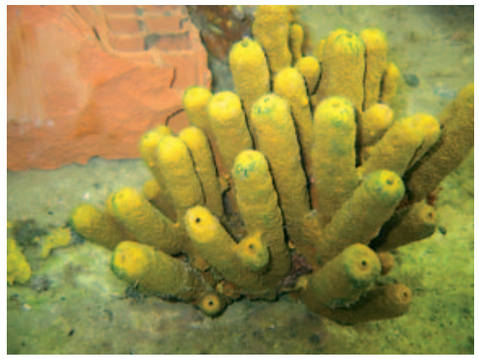
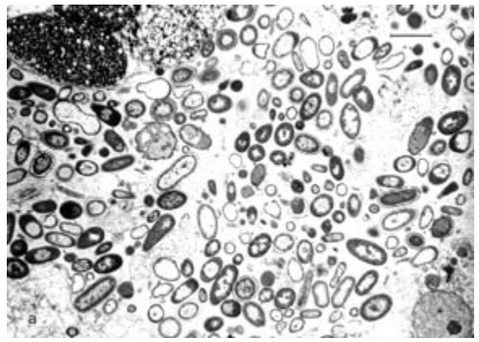
Metagenomic

Non-cultivated bacteria: the hidden diversity

Bacteria are the oldest cellular organisms on this planet. Over the course of almost two billion years, they had been the lone ruler on earth and could adapt to a staggering array of different habitats, reaching from the Antarctic ice deserts to the furnaces of deep sea volcanoes. The great diversity of the bacterial metabolism also manifests itself in a rich chemistry, from which mankind profits since about 50 years with a wide spectrum of natural product-based drugs. For the discovery of novel pharmacologically useful compounds, microorganisms are usually grown on laboratory media and screened for bioactivity. Positive candidates are cultivated in fermenters to generate biomass. The natural product can then be subjected to activity-guided isolation and structurally characterized. In this way, a myriad of novel drug candidates could be obtained from bacteria.

Today there is increasing evidence that the metabolic diversity is limited in these bacteria. For many pharmaceutical companies, efforts to identify novel substances have become economically unattractive, resulting in a decreased interest in natural products. This is an alarming situation, since there is presently no convincing alternative method to counter rising resistance rates in bacterial pathogens with new drugs.





Electron micrograph of bacterial symbionts of the sponge ***Aplysina aerophoba***. Most representatives of the highly diverse microbial consortium have so far not been cultivated in the laboratory. (Picture: Ute Hentschel)

The microbiological revolution

Several scientific discoveries have fundamentally changed our understanding of bacteria in the past decade. Molecular methods, such as ***16S rRNA analysis*** and ***in situ*** hybridization (see glossary) allow one for the first time to study bacteria without the need to cultivate them. These studies unveiled an entirely unexpected prokaryotic diversity. It became rapidly clear that virtually every habitat harbors enormous numbers of novel bacteria, compared to which the numbers of cultivated species represent a minute fraction. In some soil types, for example, these uncultivated bacteria have been estimated to represent 99.9% of all species. Not only the traditional data about biodiversity, but also our understanding about the bacterial biomass had to be drastically corrected: Studies on deep sea drill cores suggest that bacteria constitute the largest part of earth's biomass. In light of the fact that bacteria have the most diverse metabolism of all organisms, these results indicate that our efforts to investigate their natural product diversity have barely scratched the surface of what really exists. That uncultivated microorganisms could indeed be a rich source of novel natural product families is supported by the existence of numerous drug candidates that have been isolated from invertebrate animals, such as marine sponges and tunicates, and for which bacterial symbionts are suspected as true producers. The previous concern that through the in-depth study of a few microbial taxa an exhaustion

16S rRNA gene analyis
A method to determine bacterial taxonomy. The 16S rRNA gene sequence serves as a taxonomic marker.

In-situ **hybridization**
With this technique bacteria are hybridized with a fluorescence-labelled probe and visualized in a sample without the need for cultivation.

Metagenome
The entire DNA of a community of organisms.

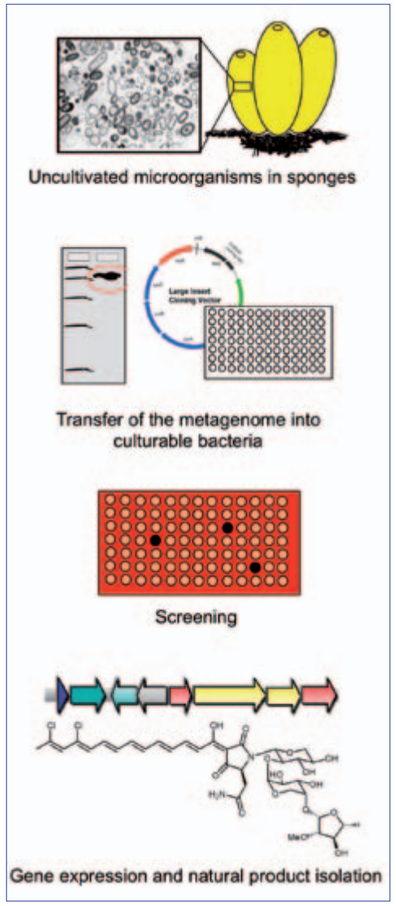
Metagenomic library
Collection of clones that carry various segments of a metagenome as foreign DNA.

Sequence-based screening
The screening of a DNA library for the presence of specific gene sequences.

of the bacterial drug potential is imminent is therefore unfounded. However, accessing this microbiological potential is not trivial and requires novel, cultivation-independent techniques, which we will introduce in the following sections.

The metagenome

The basic principle of most techniques is an isolation of the total DNA (the so-called metagenome) from environmental samples and its transfer into cultivated bacteria. If the transferred DNA contains the information to assemble a natural product, the compound might be produced by the genetically modified bacterium. The technical challenge of this strategy is that environmental samples usually contain a great number of different organisms, of which only relatively short DNA sections can be introduced into the target bacterium. Finding the genomic parts that contain natural product genes of interest therefore resembles the proverbial search for the needle in a haystack. Usually, in a first step metagenomic libraries are constructed, which are large collections of bacterial clones that contain various pieces of metagenomic DNA. To identify the correct clones, these ***libraries*** can be subjected to different screenings.



The search for bioactive compounds in uncultivated bacteria. After extraction from environmental samples, here a marine sponge, the DNA is introduced into a suitable vector and transferred into a culturable bacterium. Then, the library will screened for clones that have visibly changed properties (color, antibiotic acticity, etc.) or that carry gene families of interest.
(Picture: Ute Hentschel)

Function-based screenings

An example for a functional screening method is the observation of inhibition zones around clone colonies grown on a lawn of a test bacterium. These zones can be caused by the presence of low-molecular antibiotics that are produced due to presence of the environmental DNA. This type of screening has led to the discovery of novel antibiotics of the turbomycin series and further metabolites. At present, such studies are afflicted with several technical difficulties: metagenomic libraries usually only contain a low number of positive clones. In addition, the substances isolated to date are structurally simple, since their production is encoded by rather short DNA segments. One of the reasons for these observations is that so far mainly E. coli has been used as ***expression host***, which might not possess the necessary elements to decode DNA from distantly related bacteria and is also unsuited for the production of some pharmacologically important substance classes, such as polyketides and nonribosomal peptides. These limitations could be overcome by using additional expression hosts from various taxonomic groups. In addition, the maximal size of the DNA fragments is so far limited to about 80 kb. Often ***biosynthetic gene clusters*** of structurally complex metabolites are considerably larger and can therefore not be functionally expressed in their entirety. The development of new techniques to isolate, clone and stably express high-molecular environmental DNA is therefore an important future task.

Screening for new functions or compounds as basis for new process developments.

Sequence-based screens

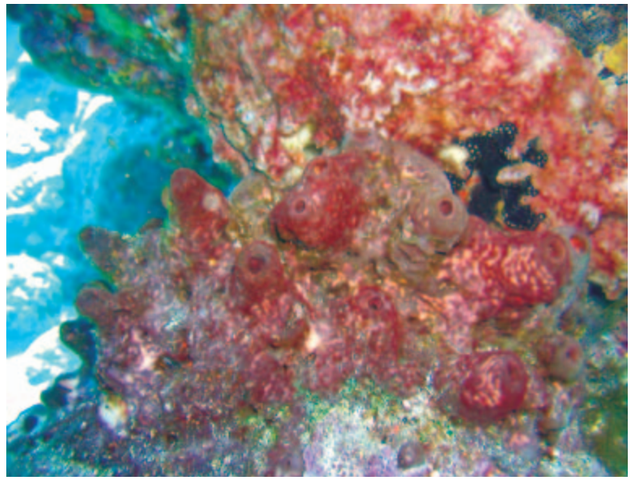
The majority of all microbial natural products is generated by only few different enzyme families. Examples for such enzymes are polyketide synthases (erythromycin, avermectins, doxorubicin) and nonribosomal peptide synthetases (β-lactam antibiotics, vancomycin, daptomycin). Library screens based on sequence similarities to such enzymes could therefore lead to the targeted isolation of genes belonging to specific natural product classes. This strategy is particularly promising for metagenomic DNA from invertebrate drug sources, since these are often associated with numerous bacteria. In marine sponges, bacteria can represent up to 40% of the total biomass. These animals can therefore be regarded as “microbial fermenters” with an untapped genetic and biotechnological potential. Invertebrate animals often contain promising drug candidates that are of suspected bacterial origin. Cloning and expressing their biosynthetic genes in a culturable bacterium could therefore result in sustainable production methods,

Modern bioreactors permit the sustainable production of basic and fine chemicals.

Function-based screening
The screening of a DNA library for biological activity, natural products or other macroscopic properties.

Expression host
A culturable organism that is used for recombinant gene expression.

Biosynthetic gene cluster
Group of adjacently located genes that are responsible for the biosynthesis of a natural product.



The rove beetle ***Paederus fuscipes*** (left) uses pederin for chemical defense against predators. The highly active antitumor compound is produced by as-yet unculturable symbiotic bacteria that live in the beetle's tissues. Similar bioactive substances are also produced by bacteria of the marine sponge ***Theonella swinhoei*** (right). Metagenomic studies have resulted in the isolation of the genes encoding the substances. (Pictures: Rupert Kellner (beetle) and Yoichi Nakao (sponge))

which are to date available for very few animal derived drugs. An example in which this strategy has been pursued is the isolation of biosynthetic genes for pederin and related antitumoral compounds, which are produced by as-yet unculturable symbionts in marine sponges and terrestrial beetles. For the first structural characterization of pederin, 24 million beetles were collected, and to date, large amounts for an in-depth pharmacological evaluation are not available. These metagenomic approaches could lead to the development of general methods to create environmentally sound production sources for a wide range of marine drug candidates. However, a targeted isolation of specific biosynthetic gene families in soil or symbiont DNA could also lead to the discovery of novel drugs. Sequencing of the isolated genes can provide insights whether they encode the biosynthesis of already known substances, of a new representative of a pharmacologically importance substance family, or even a completely novel structural type. The development of methods for high-throughput sequencing, for efficient gene analysis and for routine heterologous gene expression will in future enable us to exploit the hidden pharmacological potential of a bacterial world that is so far virtually unexplored.

Ute Hentschel and Jörn Piel

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