Molecular Strategies in the Environment

135 Years of Spell-Binding Research

Since the middle of the last century, molecular biology is its own discipline. It has its roots in microbiology, a discipline traditionally examining life processes and the role of microorganisms in different ecosystems. While today's applications of molecular biology in biotechnology and in the medical field are widely known, molecular approaches to problem-oriented environmental research are still very much in the background. This is not justified since these techniques are highly useful in the solution of current problems.

As early as the middle of the 19th century, scientists began to study the transformation of specific compounds by microorganisms. The driving force for most of the studies was the theory of spontaneous generation which proposed that life could form repeatedly and spontaneously. As an unintended consequence, these studies yielded the first insights into the metabolism of microorganisms. The French scientist Béchamp [1], a contemporary of Louis Pasteur, was as well a proponent of the theory of spontaneous generation. He examined a variety of environmental samples for their capacity to transform specific chemicals, describing for example the formation of methane from ethanol. According to his interpretation, the transformation was accomplished by microorganisms that had newly formed in his flasks; he named these organisms *Microzyma cretae.* It was Pasteur who refuted spontaneous generation with his ingenious experiments. He showed that these observations were, in reality, the growth and enrichment of microorganisms that were already present at the beginning of the experiments.

In the second half of the 19th century, the German scientist Felix Hoppe-Seyler continued the molecular strategy of environmental research. As the first Professor for Physiological Chemistry at the University of Strasbourg, he coupled the molecular understanding of biological processes to energy considerations. He recognized that every biochemical transformation yields energy that can be used by microorganisms for their growth and metabolism. Remarkable is that the now well-known classic theory of thermodynamics was just being developed (Josiah Gibbs did not introduce the term free-energy until 1878) [2]. The original approach used by Hoppe-Seyler was refined in later years.

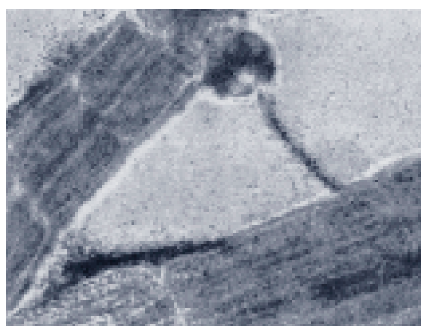


Fig. 1: Autoradiogram of epiphytic bacteria (visible as black colony, lower left) living on marine red algae. The bacteria were fed ^{14}C-glutamate as a carbon source [from 4].

Radioactive Isotopes as Markers for Metabolic Products

The next major breakthrough occurred in the 1930's and 1940's when the chemist Samual Ruben and the physicist Martin Kamen discovered the radioactive isotopes ^{11}C and ^{14}C [3]. Both scientists immediately recognized the potential that this discovery had for science. Experiments with ^{11}C proved to be difficult since this isotope has a half-life of only 21 minutes. Only when ^{14}C with a half-life of ~5700 years was used, did it became possible to follow the intermediates of biochemical transformations (even in complex systems) and to ascribe assimilation products to specific organisms within an ecosystem. With the use of microautoradiography, organisms that catalyze certain biochemical transformations in an ecosystem can be made directly visible (Fig. 1). Leading the way in this methodology were Louise and Thomas Brock during the 1960's [4].

Detection of Specific Microorganisms

During the 1970's and 1980's, biologists, particularly microbial ecologists, concentrated their research on the development of methods for direct identification of microorganisms in complex environmental samples.

Specific compounds: Some compounds are found only in very specific groups of organisms. The electron transfer coenzyme F_{420}, for example, is only found in methanogenic bacteria, with one exception. This compound is particularly interesting because it fluoresces, which allows simple detection of the organism (Fig. 2).

Immunological methods: While the detection of certain bacteria by immunological methods was already widely used in medical microbiology by the late 1970's, this technique was only starting to be employed in environmental research. Already at that time, a wide variety of markers was available for making antibodies bound to specific target organisms visible. The options included radioactive labels, enzymes that catalyze specific reactions (ELISA technique), specific heavy metals, or fluorescent

A. Zehnder, EAWAG

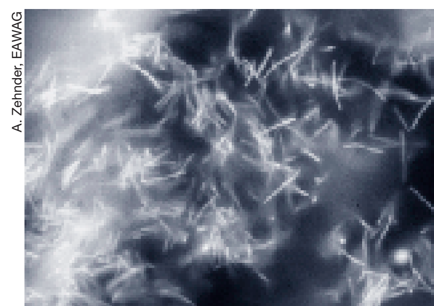
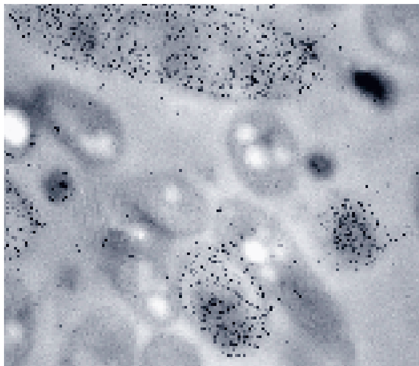


Fig. 2: F_{420}, an electron transfer coenzyme occurring almost exclusively in methanogens. UV light causes this coenzyme to fluoresce. This property allows us to directly identify methanogens in natural populations, such as *Methanobacterium formicicum* in this case.



Photographs: Wageningen Agricultural University, NL

Fig. 3: Gold colloids (black dots) bound to antibodies can be used to immunologically identify bacteria in thin sections prepared for electron microscopy. This figure shows the specific marking of *Methanosaeta concilii.* This acetate degrading organism grew in a biofilm using propionate as a substrate.

dies. Antibodies could even be used to detect individual proteins, usually enzymes, in the complex system of a single cell (Fig. 3 and 4). The disadvantage of immunological techniques is that the organisms or proteins that need to be recognized by the antibodies, first have to be isolated. They are needed for the production of the antibodies.

RNA and DNA probes: A quantum leap was achieved with the development of RNA and DNA probes in the first half of the 1980's. Two requirements had to be met before these methods could be developed: First, RNA had to be recognized and investigated as a universal indicator of relationships between organisms. Carl Woese [5] and Norman Pace [6] were the pioneers in this field. Second, DNA had to be replicated outside the cell in a test tube. In 1985, Kary Mullis [7] achieved this in his discovery of the so-called polymerase chain reaction, or PCR for short (Fig. 5). The probes are constructed such that they recognize specific RNA or DNA sections. Depending on whether the target area is a variable or a conservative region, this method can detect individual strains or entire groups of organisms. If the probes are combined with fluorescent dyes, marked organisms can be made visible directly under the fluorescence microscope. Furthermore, PCR allows us to isolate unknown gene sequences from an ecosystem and to subsequently compare them to known DNA sequences. Because we now have huge data banks on DNA sequences, chances are relatively high that the unknown sequences can be attributed to specific functions or organism groups.

What are Negative Impacts on Microorganisms?

In addition to questions about our understanding of processes, i.e., who does what, where and how, an interest in a new set of questions relating to causes and effects of environmental damage has emerged. This also includes problems around toxic chemicals that are released into the environment and can have negative impacts on organisms. The urgent need for solutions in this field has led to enormous progress in analytical chemistry. Today, a wide variety of pollutants can be measured at very low concentrations in complex matrices. In addition, our knowledge of how biological processes are controlled on the molecular level forms the basis for the development of so-called biosensors. They indicate even the lowest pollutant concentrations by the expression of specific enzymes or fluorescent proteins. Furthermore, the sequencing of entire genomes has made it possible to assemble specific gene groups and to apply them to so-called DNA chips. These chips can then be used to study the reaction of these genes to chemicals or other environmental factors.

Over the last century and a half, we have come a long way in understanding molecular processes in an ever-changing environment. The last 20 years, in particular, have been marked by a rapid expansion of our ability to apply our knowledge to the real world. EAWAG has contributed significantly to this progress, and we would like to present a few examples of our work in this issue.

The Effect and the Detection of Environmental Pollutants

Worldwide, arsenic represents one of the most important inorganic pollutants in drinking water. Millions of people in Bangladesh and Vietnam already show symptoms of arsenic poisoning. The detection of arsenic by analytical chemistry is far from trivial and nearly impossible without an infrastruc-

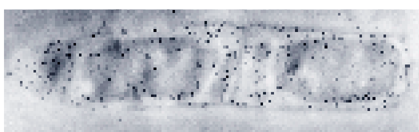


Fig. 4: *Methanosaeta concilii* in an electron microscope thin section. The enzyme carbon monoxide dehydrogenase is immunologically marked with gold colloids. The black dots appear to be evenly distributed throughout the cell, suggesting that the enzyme is soluble in the cell plasma.

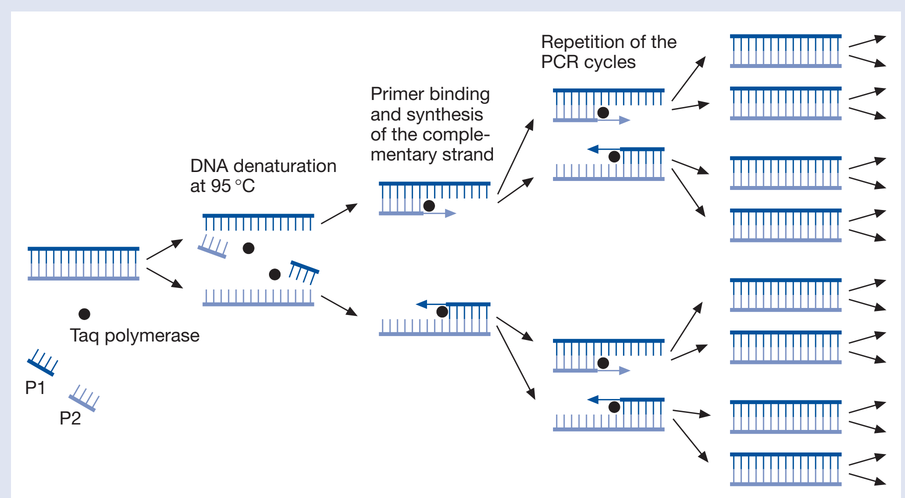


Fig. 5: Replication of a specific DNA fragment by the PCR method. The DNA to be replicated is mixed with two primers (P1 + P2) and the enzyme Taq polymerase. This mixture is subjected to approximately 30 temperature cycles in the PCR thermocycler. One temperature cycle lasts roughly 4 minutes and is made up of three steps. Step 1: the DNA is denatured at 95 °C; step 2: the temperature is lowered up to 37 °C to allow the primers to bind to the DNA; step 3: at 72 °C, the Taq polymerase synthesizes the complementary DNA strand.

ture of analytical laboratories. J.R. van der Meer and J. Stocker (p. 12) have developed a bacterial biosensor that is easy to use and can detect arsenic at the ppb level (one millionth of a gram per liter). The genetically engineered bacteria carry a so-called reporter gene which is activated in the presence of arsenic and causes the production of the corresponding reporter protein. This enzyme catalyses a reaction that releases a blue dye. Depending on the arsenic concentration, more or less enzyme is produced, which in turn releases varying amounts of dye. With a simple paper strip, even an untrained person can easily test a well for arsenic contamination.

For many years, ecotoxicology was dominated by a mostly phenomenological approach. Modern methods of molecular biology allow us to detect the expression of individual genes and, therefore, the production of the corresponding proteins in a very targeted, purposeful manner. When an organism comes in contact with a pollutant, various genes are activated, although this is not externally observable, and the organism continues its normal biochemical activities. The products synthesized by the activated genes aid in the defense against the pollutants inside the cell. In their article on page 15, B. Fischer and R. Eggen show how these defense genes can be used to detect certain pollutants; however, the response of the cell to an environmental pollutant is only half the answer. It is equally important to understand how these pollutants do their damage, i.e., we need information about how the pollutants react with biological structures. B. Escher (p. 9) and her group have investigated a number of so-called reactive chemicals for a wide range of toxic reactions and their primary toxicity mechanism.

Nitrogen Elimination and Clean Drinking Water

A group of engineers and microbiologists (see article by C. Fux and colleagues on p. 20) has examined new ways to remove nitrogen from waste water. A new method known as the Anammox process was discovered in the Netherlands and has proved to be extremely well suited for the treatment of waste water with high ammonia levels. In this process, ammonia is transformed directly to nitrogen using nitrite. A first task was to identify the microorganism responsible for this transformation. This was accomplished using specific gene probes. The practicality of the method was then tested in a pilot reactor, where successful operation cleared the way for the use of the Anammox process in full-scale reactors in wastewater treatment plants.

To date, the biological quality of drinking water is determined by cultivation techniques that are largely based on methods developed in the 19th century. The main problem of these cultivation techniques is the fact that they are rather time-consuming. Results are available after 24 hours at the earliest, often only after 72 hours. Acute contamination can, therefore, not be detected quickly. The PCR method has the potential for significant improvements in this area. Contamination could be confirmed within approximately 4 hours, leading to much shorter response times. A. Rust and W. Köster demonstrate in their article on page 18 how this method can be used in the monitoring of drinking water quality.

Gene Transfer and Genetic Diversity

Two other articles deal with evolutionary aspects. The group of J.R. van der Meer was able to demonstrate how large sections of DNA can be exchanged between bacteria. These DNA sections are referred to as "genomic islands" and can account for more than 10% of the entire genetic material. The receptor bacteria can obtain new capabilities from these transfers, such as the ability to degrade certain pollutants. This so-called horizontal gene transfer allows bacteria to accomplish important evolutionary steps with a high success rate and within very few generations.

M. Winder and P. Spaak (p. 22) examined the diversity of *Daphnia* populations in alpine lakes at varying elevations. The prevailing assumption was that genetic diversity should decrease with increasing elevation, just as the species diversity decreases at higher altitudes. Their results, however, have demonstrated that genetic diversity does not decrease but remains high even at high elevations.

"More is Different"

Rapid progress in molecular methods brings with it the danger of losing the view for the whole system but trying to explain the functioning of an entire ecosystem with a few details. It is important to use molecular strategies in trying to understand processes, and to validate this understanding at the system level. As long ago as 1972, Philip W. Anderson stated in his article "More is Different" that the dissection of a system into individual parts is not enough to help us understand the functioning of the entire system [8]. We need both, and EAWAG is fully aware of this fact.

Alexander Zehnder, microbiologist, director of EAWAG and professor for water protection and water technology at ETH Zurich. His research interests are environmental microbiology and the application of microbial processes in environmental biotechnology. He has recently also become involved in sustainable development, particularly with respect to water resources.

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