The unknown dark spot of the microcosm

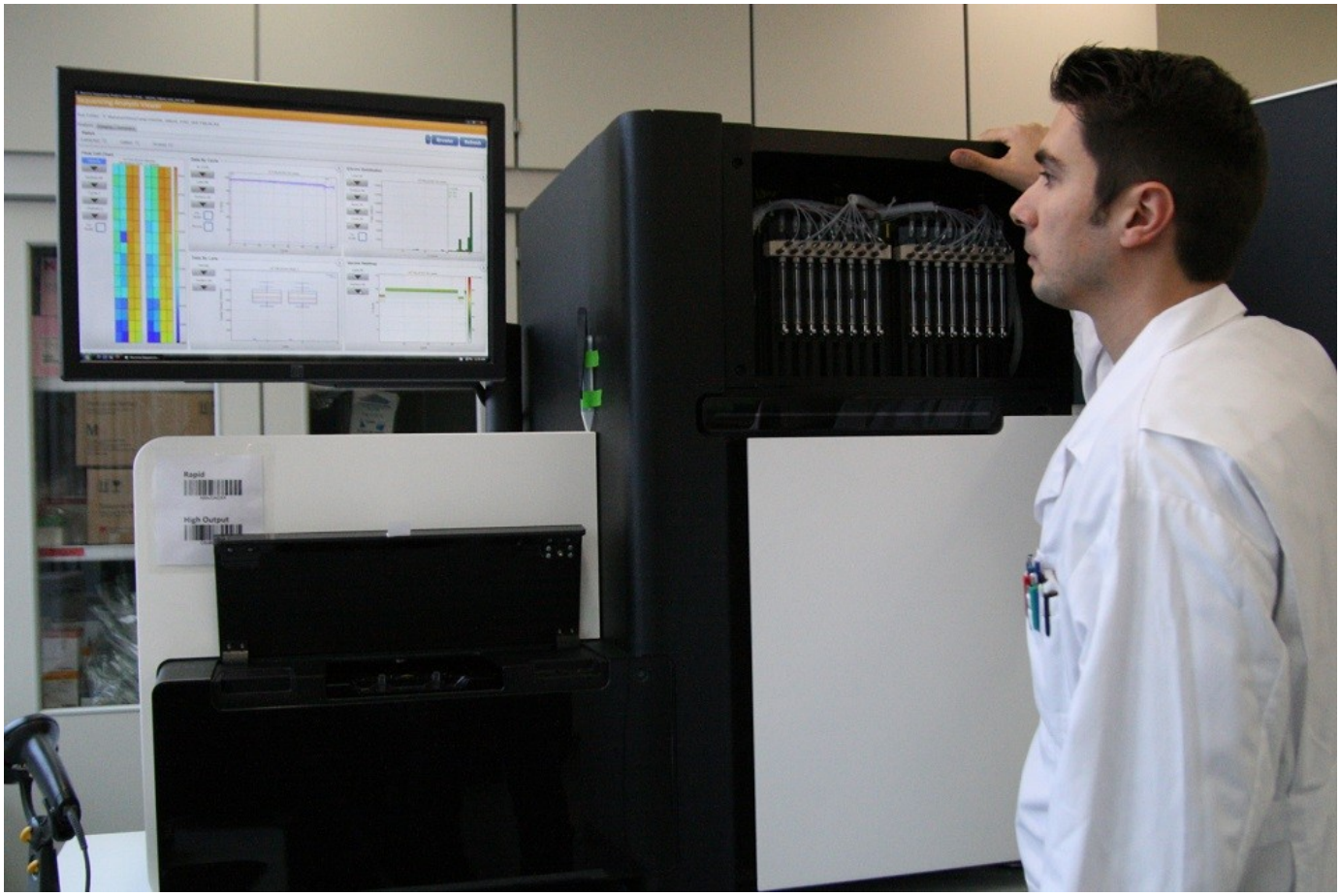
The world of microorganisms is still largely unknown. Researchers such as Kai Sohn from the Fraunhofer IGB in Stuttgart are working on decoding, analysing and gradually gaining a better understanding of the microbial genome. In their search for new enzymes and other biomolecules, both biotechnologists and pharmacologists are interested in micoorganisms, and physicians are hoping that detailed insights into the microbial genome will lead to the development of more rapid methods for diagnosing infectious diseases.

"It is assumed that about 90 percent of all microbial species cannot be cultivated, which makes their identification impossible," explains Dr. Kai Sohn, head of the working group "Functional Genome Analyses" at the Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB). He circumvents the problem by isolating and analysing all the genetic material (DNA) present in an environmental sample, i. e. the genomes of many individual organisms, known as the metagenome. Previously unknown microbial species are thus given an identity and can therefore be detected in any future environmental samples based on their genetic fingerprint.

Sohn's laboratory is equipped with a next-generation sequencing (NGS) device, a modern fridge-size sequencer that enables Sohn's team to sequence around 6 billion DNA base pairs a day. The parallel sequencing of such a large number of nucleotides was impossible around ten years ago, and instead geneticists had to focus on individual genes. It took around 13 years and US$ 3 billion to sequence the large number of 3 billion nucleotides in the human genome; this was completed and published in 2003. Meanwhile, researchers have achieved the $1000 genome, which refers to the cost of sequencing the full genome of an individual. "Today, whole-genome sequencing has become routine and opens up new fields of application," says Sohn.

In order to identify the individual species of a microbial community, Sohn's team cuts DNA into more manageable fragments. The 10 to 50 million or so fragments are sequenced and subsequently assembled into whole genomes of individual microbial species, which is a relatively tedious procedure. "It will not be possible to assign the large majority of our sequences to the human or other reference sequences stored in the databases," says the molecular biologist. This is because the researchers are dealing with previously unknown species.

In the right order – first sequencing and then translation



Sohn's colleague, Dr. Christian Grumaz, standing in front of a next-generation sequencer.
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Molecular biologist Kai Sohn checking a sequencing chip that can be used to simultaneously sequence up to 1.6 billion DNA fragments.

Sohn's team has identified more than 200 different microbial species in the fermentation tanks of biogas facilities. The composition of the microbial communities differed from one fermentation tank to another, depending on whether the substrate was corn or amaranth. The microbe composition also changed in the course of biogas production. "But this says nothing about what these organisms can or cannot do," says the biologist. It cannot be deduced from the order of the DNA "letters" whether or not the organisms make proteins that are useful for producing biogas.

In the same way Germans can read an English text without necessarily understanding what they are actually reading, molecular biologists also have to translate the DNA letters into something they can use to deduce function, etc. They use specific software programmes to screen the complete genome for known patterns in order to be able to predict gene functions. The researchers identify active genes by sequencing all the gene transcripts in a microbial community. This is referred to as a metatranscriptome. The comparison with organisms known to be involved in biogas processes enables the researchers to reconstruct the metabolic pathways. "We hope to control the biogas process in order to achieve consistently high yields. We can do this by adding suitable microorganisms," says Sohn.

Functional genome analysis is also of major interest to pharmacologists and chemists. An in-depth understanding of a microorganism's blueprint helps the researchers to manipulate it specifically for the purposes they want to use it for. The researchers at the Fraunhofer IGB have discovered around nine new enzymes of the P450 family in bacterial cultures, and expect that these will facilitate certain synthesis steps that are important for drug and fine chemical production in the pharmaceutical and cosmetic industries. Chemical synthesis of these substances in the laboratory is relatively complicated.

DNA in the blood of infected people enables the identification of the causative agent

The diagnosis of infectious diseases also benefits from next-generation sequencing. Sohn's team is currently developing a rapid test aimed at identifying the causative agent of sepsis within one to two days based on the free DNA in an infected person's blood. At present, microbiologists need to cultivate the pathogen for several days before they can identify it, with varying degrees of success. However, physicians require an early diagnosis to be able to treat the infection before it becomes a life-threatening condition. For tests that are based on polymerase chain reaction (PCR), the molecular biologists need to know which microbial species they are looking for. "We sequence the complete free DNA in a patient's blood without prior hypothesis, then assign the DNA to individual organisms and we can even say how often a specific species is present," says Sohn summarising the advantages of the new method.

"Since 2005 when the new sequencing technologies entered the market, they have caused an absolute revolution that dwarfs digital development," says Sohn, visibly impressed. "Data collection no longer presents a problem for us. Nowadays, it is the interpretation of data and bioinformatic analyses that is causing the bottleneck," says Sohn.

Article

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Further information

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- Functional Genome Analyses – Fraunhofer IGB

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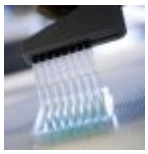

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